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Shining Light on Manure Improves Livestock and Land Management

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Technical Bulletin
SANG-2010-0250
July 2010

The use of near-infrared reflectance spectroscopy (NIRS) of feces to determine the nutrient content of the diets of grazing animals began about 20 years ago. This volume provides the history of the development of fecal NIRS in grazing animal nutrition, the current state of the science and potential new applications for grazing livestock.

Shining Light on Manure Improves Livestock and Land Management

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First edition, first printing.
Printed in the United States of America.
Brown Printing, Inc., Jefferson City, Missouri http://brownprint.com.

DEDICATION

Jerry W. Stuth
1947 - 2006

Jerry Stuth pioneered the use of fecal profiling with near infrared reflectance spectroscopy (NIRS) to determine the nutritional value of forage intake by free ranging animals. He also was recognized nationally and internationally as a leader in the development and application of a wide array of information technology in grazing land ecosystems.

Jerry was at the forefront of development of a method where feces defecated in a pasture can be scanned with NIRS and diet crude protein and digestible organic matter predicted accurately. When coupled with the NUTBAL (Nutritional Balance Analyzer) nutritional management software that he developed, it can be determined if the animal has a deficit or excess in protein and energy consumption, translate that to weight gain and loss and determine least cost solutions of feed inputs to correct the problem.

In 1994, Jerry established a national service lab at Texas A&M University, the Grazingland Animal Nutrition Lab, or GAN Lab as it is known. The GAN Lab processes up to 10,000 samples a year for producers from 45 states and 14 countries. A survey of producers using the system showed an annual benefit of $35 per exposed cow. Between 1997 and 2003, the National Resources Conservation Services (NRCS) funded between 4000 and 7000 samples per year for producers working in grazing land management. Jerry was instrumental in establishing labs in East Africa, Argentina, and Mongolia. An Afghanistan lab is in progress. He collaborated with Australians working in NIRS, one of whom, David Coates, is a

contributor to this monograph. Jerry and Doug Tolleson were responsible for introducing the technology into northeastern Mexico and working with Dr. Ricardo Silva and his students at Universidad Autonoma Agraria António Narro, several of which have entered graduate programs in the U.S. to pursue work in nutrition. The GAN Lab has supported 40 undergraduate and 12 graduate students working on degrees at Texas A&M University.

In 1988 Jerry provided leadership in the Ranching Systems Group at Texas A&M who developed a comprehensive computerized resource planning system for grazing lands that was adopted as the official planning tool by USDA NRCS. The original program, Grazing Lands Applications (GLA) has been transformed into an advanced package called Grazingland Spatial Analysis Tool (GSAT). It forms the foundation for planning activities in Environmental Quality Incentive Program (EQUIP), Conservation Reserve Program (CRP), and other USDA agricultural assistance programs. GSAT includes a nutritional analysis component that uses the Nutritional Balance Analyzer (NUTBAL) nutritional management program based on fecal samples and the use of NIRS.

Jerry developed the first livestock early warning system (LEWS) that delivers 90-day forecasts of impending shortfalls in forage production for East Africa, parts of the U.S. and now Mongolia every 7 to 16 days. The technology, based on the PHYGROW (Phytomass Growth Simulator Model) forage production model developed by a team headed by Jerry, serves over 400 ministries and non-governmental organizations (NGO) in East Africa.

Given the success of the East African LEWS, the U.S. Agency for International Development (USAID) selected the research group led by Jerry to establish the first early warning system for drought in east Asia. This team is also introducing new portable NIRS fecal profiling technology to improve livestock nutrition. The system also uses the new U.S. Air Force Weather system for snow and ice disaster mapping. A similar system is in early stages of development in Afghanistan for western Asia. In the U.S., Texas, Oklahoma, New Mexico, Louisiana, Arkansas, Montana, Wyoming, West Virginia, and Oregon have varying degrees of coverage.

Jerry was leader of the team that developed the Forage Risk Assessment and Management System (FRAMS). It was the first on-ranch early warning system allowing ranchers to record their own vegetation, weather, and grazing practices while the system provided both biological and economic feedback on how to make adjustments in stocking practices. FRAMS users can also use NIRS for nutritional assessments within the system. FRAMS has been pilot tested in Texas, Wyoming, New Mexico, and Pennsylvania and is currently being expanded to additional producers in Texas through an NRCS grant.

Jerry was a highly respected professor and had an outstanding record of research and publications in his academic role. He chaired the graduate committees of over 60 Masters and PhD students. Beginning in 1975, Jerry taught over 2000 undergraduate and graduate students at Texas A&M. He administered over $35,000,000 in grants over the past twenty years, an

outstanding accomplishment and testimony to the professional respect that he enjoyed with major research funding organizations throughout the U.S. and the world. He also authored or co-authored 92 refereed journal articles, 24 books and book chapters, many proceedings, agency publications and software user's guides, and he developed and released 22 software programs.

Jerry served as major professor for a group of scientists working in NIRS and across a variety of research projects. Bob Lyons, another author for this monograph, was Jerry's first graduate student working with NIRS technology. Students who followed Bob Lyons and developed F.NIRS calibration equations include Eneas Leite (goat), Sarah Ossiya (East African diet quality), Kosi Awuma (West African cattle, sheep and goats), Negusse Kidane (donkeys), Hong Li (U.S. sheep equation), Scott Keating (elk), Scott Showers (deer) and Evan Whitley (protein fractions in cattle rumen). Work is currently underway by Erin Weidower on a panda equation for the Memphis, Tennessee zoo.

It was my good fortune to work closely with Jerry Stuth over a 30-year period in research and teaching endeavors. I traveled over much of the U.S. and the world with him and saw his passionate pursuit of the technology that is being showcased in this publication. Few scientists have devoted as much of their professional lives to expanding our technical knowledge and making it applicable to such a wide range of the earth's natural grazing land resources as Jerry Stuth.

In 2009 the Society for Range Management bestowed its highest honor, the Fredrick G. Renner Award, to Jerry in recognition of his many contributions to the art and science of range management. He is in an elite class of individuals from our profession who are the "tall trees in the forest."

Wayne Hamilton

FOREWORD

The difficulty of monitoring the nutrient and botanical diet composition of free ranging herbivores as well as other physiological parameters related to their wellbeing has always limited the development of technologies for increasing the efficiency of livestock production or managing the ecological impact of their foraging behavior. Fecal near infrared spectroscopy (F.NIRS) is a technique with the potential to improve our ability to measure the dietary and physiological characteristics of grazing animals. However, it is not without its detractors. The authors admittedly are proponents of the use of NIRS for rangeland management but have tried to present an objective overview of past and current research on the topic. The purpose of this publication is to provide an overview of F.NIRS for ranchers, agency personnel, and researchers so that those without a technical background in spectroscopy can evaluate its potential applicability to their needs.

This publication is the result of a symposium held in 2007 at the annual meeting of the Society for Range Management. The symposium and this publication resulted from the confluence of two events: 1) the development of a critical number of scientists involved in this research topic and 2) the untimely loss of Dr. Jerry Stuth, a pioneer of the technology. The authors of this publication represent most of the professionals involved in F.NIRS at the time of the symposium. We felt that it was time to collect, in one publication, an overview of the existing knowledge, while simultaneously honoring our late colleague.

The primary focus of the symposium and this publication are applications of near infrared reflectance spectroscopy (NIRS) of feces to predict various parameters of interest relative to the nutritional status and ecological impact of free grazing herbivores on rangelands. Although we hope this publication will be of value to people with a wide array of interest and understanding of NIRS, the target audience is professionals who might benefit from the use of fecal NIRS, but whose adoption of the technology is limited by a lack of knowledge of the benefits and limitations of F.NIRS.

Special appreciation is also expressed to the following for providing technical reviews of this material:

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Acronym Guide

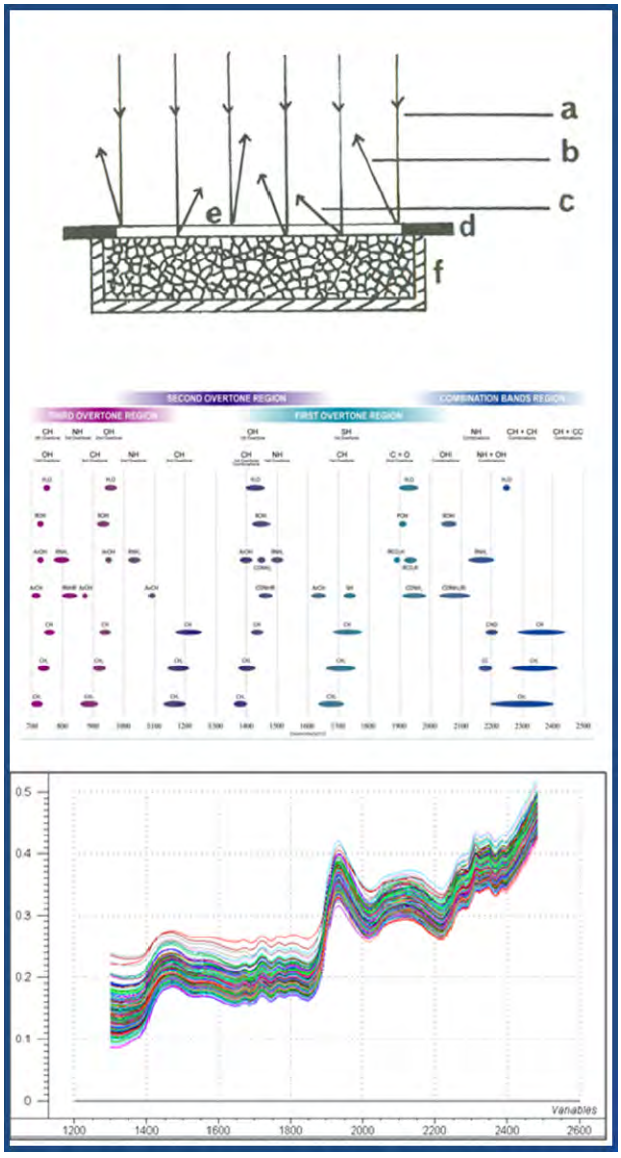
ADF	Acid detergent fiber
BCS	Body condition score
BLM	Bureau of Land Management
CP	Crude protein
CRP	Conservation Reserve Program
CSIRO	Commonwealth Scientific and Industrial Research Organisation
DM	Dry matter
DMD	Dry matter digestibility
DOM	Digestible organic matter
EF	Esophageal (oesophageal) fistulated
EQUIP	Environmental Quality Incentive Program
F.NIRS	Fecal near infrared spectroscopy
FRAMS	Forage Risk Assessment and Management System
GAN	Grazingland Animal Nutrition
GLA	Grazing Lands Applications
GSAT	Grazingland Spatial Analysis Tool
ICACG	Israel Council on Animal Care Guidelines
LEWS	Livestock Early Warning System
ME	Metabolizable energy
MPLS	Multiple partial least squares
NDF	Neutral detergent fiber
NGO	Non-governmental organization
NIRS	Near infrared spectroscopy
NOAA	National Oceanic and Atmospheric Administration
NRCS	Natural Resources Conservation Service
NUTBAL	Nutritional Balance Analyzer
PHYGROW	Phytomass Growth Simulator Model
r^2	Simple coefficient of determination
R^2	Multiple coefficient of determination
RMSED	Root Mean Squares of Differences
RMSEP	Root Mean Square Error of Prediction
RPD	Ratio of standard deviation of prediction to standard deviation of samples
SE	Standard error
SEC	Standard error of calibration
SECV	Standard error of cross validation
SED	Standard error of differences
SEM	Standard error of the mean
SEP	Standard error of prediction
SNV	Standard normal variance
USAID	US Agency for International Development

Table of Contents

Chapter 1. Primer on Near Infrared Spectroscopy

John Walker

Objectives: To describe near-infrared reflectance spectroscopy (NIRS) and how it works to convert light reflected from a sample to useful information about nutrients or botanical composition of a sample, and the statistics used to find how reliable it is.



Key Points

- Light can be absorbed by the chemical bonds in organic compounds and reflected to a detector. This reflectance allows many compounds and physical properties to be found in a single near-infrared reflectance spectroscopy (NIRS) analysis.
- Chemometrics is the field of extracting information from multi-variable spectral data using statistics and mathematics. Chemometrics was used to develop all of the calibrations in this publication.
- Calibrations, which change the reflected light to useful information are classified as:
  - Direct - to predict materials that directly absorb NIR radiation.
  - Indirect – to predict materials that do not absorb but are correlated with organic molecules that do absorb NIR radiation.
  - Derivative – developed when spectra are collected on one material and used to predict a property of another material.
- Calibration data set structure is a critical consideration but indications are that the derivative calibrations used in F.NIR may be valid across a broader array of sample variation than direct and indirect calibrations used for most agricultural products.
- Calibration equations are evaluated using statistics that estimate the precision and accuracy of the predictions relative to their laboratory value. Formulas for these statistics are provided in this chapter.

Principles of Spectroscopy

Spectroscopy is the study of the interaction between electromagnetic radiation and matter as a function of wavelength (λ). Electromagnetic radiation is absorbed by chemical bonds when the energy of a light photon is equal to the energy difference between two vibrational and rotational states of a chemical bond. Energy and wavelength are related and convertible from one to another. Thus the wavelengths of absorbed radiation are unique for each molecule; intensity of absorption is proportional to the concentration of molecules and therefore can be interpreted to understand the composition of a substance. The initial absorptions by organic molecules are in the infrared region. They are the fundamentals that result in narrow absorption peaks and can be directly interpreted to determine the composition of a substance.

Absorptions in the near-infrared region (780 – 2500 nm) by organic molecules are due to overtones or combination bands primarily of O-H, C-H, N-H and C=O. Overtones can be thought of as harmonics and represent whole integer multiples of the much stronger fundamental absorption frequencies found in the mid-infrared region (2500 – 50,000 nm). Combinations arise from the sharing of NIRS energy between two or more fundamental absorptions. Because NIRS radiation has more energy than the mid-infrared region where fundamentals are located, longer pathlengths are possible and special sample preparation is not necessary. However, overtones and combinations create complex NIRS spectra with broad absorption bands that are composed of multiple narrow, overlapping absorptions. NIRS spectra are much more complex than they appear and were not useful until the advent of high-speed computers and multivariate algorithms to convert complex spectra to useful information.

NIRS spectra can be measured as transmittance (light energy passing through a liquid) or reflectance (light energy reflected off a solid). The research presented here is based primarily on reflectance spectra, and this primer will concentrate on this type of spectra. Light energy directed at an uneven or granular surface is either specularly or diffusely reflected. Specular reflectance is reflected directly from the surface and contains no information relative to chemical bonds. Other portions of the spectra are absorbed by the molecular bonds in the sample before the remaining energy is reflected back to the detector. The radiation that enters the sample and is reflected back is termed diffuse reflection because it becomes diffused by random reflections, refractions and scatter at further interfaces inside the sample. This reflected energy is affected by particle size of the sample, and the observed spectrum contains information about both the chemical and physical nature of the sample. The nature of diffuse reflectance allows multiple constituent and physical properties to be determined from a single diffusely reflected spectrum.

Chemometrics

As indicated previously NIRS spectra are complex and their interpretation is not straight forward. Chemometrics is the field of extracting information from multivariate chemical or spectral data using tools of statistics and mathematics. In spectroscopy, the principal application of chemometrics is in calibration. The variable that calibrations are developed for are referred to as a constituent or an analyte. The concentration of the constituent is determined by a standard analytical procedure.

Diffuse reflectance results in baseline shift (i.e., higher peaks that increase multiplicatively as absorption increases at the longer wavelengths, and with larger particle sizes). A variety of data pretreatment techniques are employed to correct for such problems and to help differentiate the overlapping peaks prior to calibration. Multiplicative scatter correction, standard normal variate and detrending are techniques used to correct for the multiplicative baseline shift caused by light scattering. Derivatives are also commonly used to remove baseline shift and define spectral peaks. First derivatives remove additive baseline shift and second derivatives remove linear baseline shift and can help separate overlapping absorption peaks. Derivatives tend to reduce signal relative to noise, and this is in part, compensated for by adjusting the gap between points over which the derivative is calculated and the number of points in a moving or boxcar average over which the data is smoothed prior to calculating the derivative. A common notation for describing mathematical treatments that use derivatives and smoothing is D,G,S1,S2 where:

D = derivative number
G = gap or number of data points over which the derivative is calculated
S1 = number of data points in moving average before calculating the derivative
S2 = the number of data points in moving average for a second smoothing (rarely used)

These data pretreatments are mentioned to give the reader an appreciation for their use because they are commonly used and were used prior to all calibration in all subsequent chapters. While there are theoretical considerations for data pretreatments, they are typically applied empirically to determine the best calibration.

Calibration is achieved by using the spectra as multivariate descriptors to predict concentrations of constituents or physical properties of interest using statistical approaches such as multiple linear regression, principal components analysis, partial least squares or discriminate analysis for classification. Because of the high degree of correlation between adjacent spectra and the ability to optimize factors for their correlation to constituents while maintaining orthogonality between factors, partial least squares is the most popular method for calibration. Without the mathematical and statistical methods available, few of the quantitative applications would have been developed. But the perceived complexity of these methods and the potential they offer for abuse has hindered the adoption of NIRS in some fields.

Calibrations can be classified as direct, indirect, and derivative. Direct calibration occurs when the constituent of interest contains chemical bonds that absorb the electromagnetic radiation. Indirect calibration is the ability to determine constituents such as inorganic materials (e.g., salts that do not absorb in the NIRS region). In this case, calibrations are possible because the constituent of interest covaries with one or more organic molecules that do absorb in the NIRS region. Derivative calibration, a term which has not been used previously in NIRS literature, is when spectra are collected on one material and used to predict a property of another material. Thus the material from which spectra are collected is wholly or partially derived from the material from which the constituent data is collected. For instance, fecal material from livestock has been measured to predict the chemical

and botanical composition of the diet from which it was partially derived.

Equally important as the mathematical algorithms for developing NIRS calibrations is the structuring of calibration sample sets. Improperly structured calibrations will "overfit" the equation, lack robustness, and give overly optimistic expectation for this technique. Cross-validation involves sequentially withholding samples from the calibration set and using the withdrawn samples to validate a model developed from the reduced calibration set. This procedure is often used to determine the appropriate number of factors or terms in the model, which is the number that minimizes the cross-validation prediction errors. Cross-validation is also used as a substitute for independent validation when independent samples are not available for validation.

Data Set Considerations

In situations where changes are expected to happen, for instance, when a calibration is used for a new season or when samples are taken from another place, it is very important to check calibrations carefully (Naes et al. 2002, p. 201). This is almost always the case for F.NIRS because by the time feeding and/or grazing trials to create fecal diet pairs are completed and calibrations developed, the season will change and these calibrations are used for locations that are different than the source of feeds for the calibration trial. Validation samples should cover the range of variability anticipated in future samples. This, however, is usually impossible to guarantee, and additional validations are recommended to detect changing situations and revise calibrations (Hruschka 2001, p. 43). Monitoring is the use of standard laboratory analysis at regular intervals on small sets of check samples. Prediction errors are plotted on a control chart and if errors exceed the established control limits for a set of samples, the need for recalibration is indicated. Recalibration is the process of adding new samples to the existing calibration set and deriving a new calibration equation. This standard procedure for NIRS analysis of most agricultural materials is generally not practical for F.NIRS because of the difficulty of obtaining a representative diet sample. However, bite count estimates of diet composition (chapter 6 this volume) may provide an alternative for some situations. The difficulty of monitoring F.NIRS calibration equations emphasizes even more strongly than the empirical nature of this procedure the importance of creating robust calibrations and validation procedures that do not overstate the expected precision and accuracy of applying calibration equations to independent samples.

Factors affecting F.NIRS have not been well studied but for a commodity such as wheat, the variables most likely to affect the wheat are growing season, growing location, planting time, irrigation and fertilizer practices, wheat variety, fungal and insect disease, and weather. For a more complex commodity such as forage, plant species and stage of maturity should be added. For an even more complicated commodity such as a feed mix, the individual components of the mix should be considered. All variables should be included at least three times in any calibration, and accumulation of sufficient samples for a stable calibration is often the most critical aspect (Williams and Norris 2001). Growing seasons introduce variation, and normally 5 – 6 growing seasons are required to represent grains adequately (Williams 2001). Routine use of NIRS to determine the composition of agricultural products relies on extensive calibration sets to develop robust calibrations that represent the variability expected in future determinations. Because of the difficulty in obtaining

fecal samples from diets with known concentrations of a target plant, we are now just beginning to understand the requirements for F.NIRS calibrations. However, there is some indication based on the limited sample size compared to calibrations of agricultural products that F.NIRS calibrations may be more robust than would be expected based on experience from calibrations of standard agricultural products. Chapter 2 provides some insight into why this may occur for F.NIRS.

Calibration and Validation Statistics

Several statistics generally are used to evaluate the usefulness of calibration equations. These statistics measure either the precision, the accuracy, or in some cases both precision and accuracy of predictions. The statistics described are calculated using the lab value of the sample as the dependent or y value and the NIRS predicted value as the independent or x value. Technically such designation is known as inverse calibration (Naes et al. 2002, p. 11-12) and some chemometric software reverse the order of these two variables but the only statistic this affects is the slope. The above order for x and y variables was used here because it is more intuitive to most readers.

The simple coefficient of determination (r^2) is the proportion of the total variation in the constituent or laboratory values explained by the NIRS predictions. It is an indicator of precision and in F.NIRS it is possible to have high r^2 values and low accuracy. Bias is the mean difference between the lab value and the NIRS predicted value and can result from a slope that deviates significantly from 1, a y-intercept that differs significantly from 0, or certain combinations of these two. Bias and slope are indicators of accuracy.

Standard error of differences (SED) and root mean square of differences (RMSED) are measures of the actual prediction error (Table 1). SED is corrected for bias and thus is a measure of precision and RMSED is not corrected for bias and is affected by both accuracy and precision. The terminology for SED and RMSED is dependent upon the source of the differences used in the calculation. Typically SED is calculated for errors in the calibration model, cross-validation error and error in determination of independent validation samples and is referred to as standard error of calibration (SEC), standard error of cross-validation (SECV) or standard error of prediction (SEP), respectively. Similar terminology can be used for RMSED. Two times the RMSED or SEP (when bias is small) provides an approximate 95% confidence interval for NIRS determinations. The ratio of the standard deviation of prediction to standard deviation of the samples (RPD) enables the evaluation of SEP in relation to variability of the reference samples. The formulas and guidelines for interpretation of these statistics are presented in Tables 1 and 2, respectively.

Table 1. List of statistical nomenclature, symbols and formulas used to evaluate calibration and validation of NIRS chemometrics**.**

Statistical Nomenclature	**Symbol**	**Statistical Formula**
Correlation coefficient	r	$\frac{\Sigma(x \cdot y) - [\Sigma x \cdot \Sigma y)/N]}{\sqrt{(\{\Sigma x^2 - [(x)^2/N]\} \cdot \{\Sigma y^2 - [(\Sigma y)^2/N]\})}}$
Simple coefficient of determination	r^2	r^2
Standard Error of Differences	SED	$\sqrt{\Sigma(x - y - BIAS)^2 /N - 1}$ where: $BIAS = \Sigma(x - y) /N$ **OR** $\sqrt{\Sigma(x - y)^2 - \{[\Sigma x - y]^2/N\}/N - 1}$
Standard Error of Calibration	SEC	SED formula where: x = F.NIRS prediction y = laboratory constituent value
Standard Error of Cross-Validation	SECV	SED formula where: x = F.NIRS prediction w/o cross-validation samples in the model y = laboratory constituent value for cross-validation samples
Standard Error of Prediction	SEP	SED formula where: x = F.NIRS prediction y = laboratory constituent values for independent samples
Root Mean Square Error Difference	RMSE	$\sqrt{[\Sigma(x - y)^2/n]}$
Standard deviation of y	SDy	$\sqrt{[\Sigma(y - \bar{y})^2/n-1]}$
Ratio of SEP to SD	RPD	SDy/SEP
Bias	Bias	$\bar{x} - \bar{y}$
Slope	b	$\Sigma xy/\Sigma x^2$

Table 2. Evaluation of validation statistics based on recommendations of Williams (2001).

r^2 Value	**RPD Value**	**Interpretation**
< 0.49	0.0 – 2.3	Not useful
0.50 – 0.64	2.4 – 3.0	Very Rough Screening
0.65 – 0.81	3.1 – 4.9	Screening
0.82 – 0.90	5.0 – 6.4	Quality Control
0.91 – 0.96	6.5 – 8.0	Process Control
> 0.97	> 8.0	Any Application
Slope value		
0.95 – 1.05		No adjustment necessary
0.80 < 0.95 or 1.05 > 1.15		Slope adjustment useful
< 0.80 or > 1.15		Calibration is probably very sample dependent and cause of deviation should be investigated

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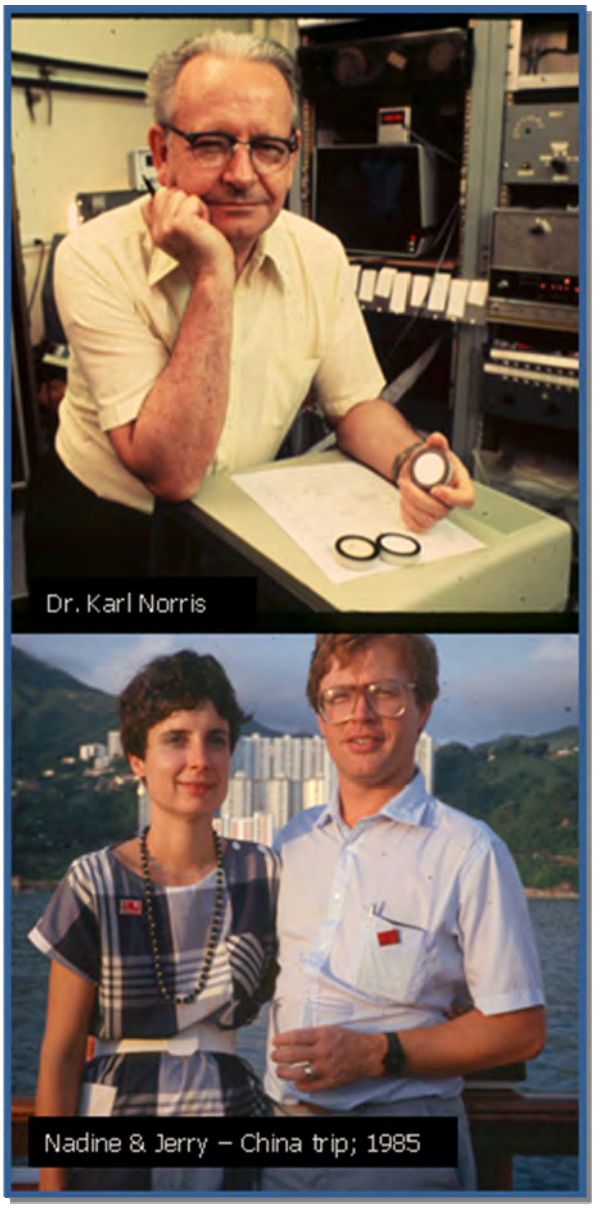
Chapter 2. Historic Overview for Fecal NIRS Analysis

Sam Coleman

Objectives: To describe the history of near-infrared reflectance spectrometers (NIRS) for finding the composition of agricultural products and how this technology grew beyond the nutritional evaluation of forages to using feces to determine diet quality. Feeding trial limitations and how that affects F.NIRS is also discussed.



Key Points

- Investigation of near-infrared spectrometers for agricultural applications were begun by Karl Norris in the 1950's to measure moisture and fat in agricultural products.
- The standard for assessing nutrient value of forages for ruminant livestock is the direct measurement of intake and digestibility in feeding trials.
- Methods for measuring forage digestibility without using costly feeding trials are available, but simpler methods for measuring intake are not.
- NIRS of forages were shown to predict digestibility and intake better than equations based on chemical contents.
- The next step was to collect spectra from fecal samples to predict diet nutrient composition. This showed that crude protein could be predicted best followed by digestibility, but prediction of intake by F.NIRS is uncertain.
- Increased confidence in F.NIRS findings of diet nutrient content and intake requires an understanding of the inherent variability of standard feeding trials.
- Development of calibration sets is a critical issue needing one of two approaches:
    - Narrowly defined tightly controlled samples with high accuracy for specific applications.
    - Broad array of samples from a variety of backgrounds and seasons with lower accuracy but broader application.

Historical Review of NIR Historical Spectroscopy

No history of the use of F.NIRS for analyzing feces could be complete without first considering the use of the near-infrared energy spectrum in general. Near-infrared is that part of the electromagnetic spectrum consisting of energy waves slightly longer than those visible to the naked eye. Wavelengths of energy range from several meters in length (nuclear magnetic resonance and radio) to less than 10^{-2} nanometers (cosmic rays). For reference, the human eye can perceive colors that are positioned between about 300 to 700 nm and the near-infrared region lies between 780 and 2500 nm. When molecules are irradiated with an external source of energy, they have the potential for energy changes (Murray and Williams 1987). Upon irradiation, the energy level is elevated from its ground state to a higher level, generally related to the sum of vibrational and rotational changes. Coblenz (1905) studied and reported on the vibrational and rotational characteristics of atoms and groups of atoms of different molecules in response to irradiation with near-infrared light. In the 1950s, Kaye (1954) described the instruments and the theoretical basis for NIR spectra in rather simple molecules, which led the way to future activities. Later in the 1950s and 1960s, Karl Norris and associates began to utilize computerized spectrophotometers to quantitatively determine moisture and fat in agricultural products (Hart et al. 1962; Norris and Hart 1965). The latter paper described a direct prediction (analysis) through the use of diffuse reflectance, a phenomenon that became very popular because of the minimal sample preparation. Diffuse reflectance occurs when a sample is bombarded with electromagnetic energy (usually from monochromatic light). Part of the energy is reflected directly with no interaction with the sample (specular reflectance). The remaining energy interacts with the physics and chemistry of the organic components of the sample (mostly molecules composed of C, H, N, and O), but eventually exits the sample at various angles (diffuse reflectance). Electronic detectors can precisely measure the energy reflected, and if done at different wavelengths, provides a spectrum of reflected energy. The relative differences of reflected energy at different wavelengths are characteristic of the chemical bonding characteristics of the sample and can be used to estimate the chemical, physical, and (by association) biological attributes. With this technology, samples of seeds (or feed) could be analyzed directly without extractions, physical, or chemical preparations. Norris et al. (1976) showed that the technology could be used to measure chemical composition, intake and nutritive value of forages with reasonable precision using only the dried, ground sample obtained from digestion trials conducted throughout the USA.

Following the Norris et al. (1976) paper, many forage scientists became interested in using NIRS, probably because the current techniques for estimating forage quality were so laborious, costly, and rather inadequate in terms of precision. Furthermore, it was a non-destructive procedure in that the sample was not consumed during analysis. In 1978, a group of USDA-ARS scientists met to investigate the feasibility of developing a network of collaborators equipped with similar instruments to further investigate the technology and to develop software and procedures to facilitate its transition to general use. The group expanded to include researchers from universities in the US and internationally. A handbook was published

and later revised with supplements (Marten et al. 1989). Roberts et al. (2004) provide a comprehensive review of the use of NIRS for forage analysis.

Approach to Forage Analysis for Nutritive Value

Animals eat to satisfy nutrient requirements for maintenance, production, and work. Performance is largely determined by the most limiting nutrient. Ruminant animals depend largely on forages, whether harvested and fed or grazed. Major limits to optimal production during some part of the year are caused by nutrient deficiencies. The most common are digestible (or metabolizable) energy and crude protein. Minerals are quite cheap and are normally supplied free choice in a supplement. Therefore with forage fed animals, knowledge of the intake of digestible energy and protein should be useful for predicting potential performance. If a sample of the diet is available (stall fed animals), nutrient composition (gross energy, crude protein, fat, fiber) can be obtained from laboratory analysis. Direct measurement of the amount eaten and *in vivo* determination of digestibility are the *de facto* standards for determining the two aspects of forage quality - intake and digestibility. However, animal trials are laborious, time consuming, costly, and require a substantial amount of the test feed; therefore, they are totally impractical in screening of genetic resources (Castler 1997). Also, there are large sources of error associated with the measurements due to animal preference and behavior, health, sampling, wastage and many other factors. Standard errors of determination (residual among animals fed the same feed) often exceed 5% of the mean for digestibility and up to 20% for intake (Coleman et al. 1999; Moore et al. 2007). These high errors might also reflect our ineffectiveness in properly replicating and conducting digestion trials as much as they do inherent variability. High costs have pushed investigators to use collection periods that are too short and with minimal replication. Indirect methods are necessary, and techniques to assist plant breeders must be developed if progress in developing countries is to move toward that observed in the developed world. Bioassays are available for estimating digestibility such as *in vitro* (Tilley and Terry 1963) and *in situ* (Orskov et al. 1988) methods. Whereas these bioassays have enhanced our ability to estimate digestibility on large numbers of samples, a similar assay to estimate intake has been more elusive. For these reasons, many attempts have been made to predict intake and digestibility from simple chemical values (Rohweder et al. 1978). Most of the attempts resulted in empirical equations based on one or more chemical components and a finite population of forage samples (e.g., alfalfa). Such equations typically are useful only for forage samples of the population from which they are based (same species, year of harvest) and variability often is obtained primarily from differences in maturity. Failures result because variation in the statistical relationship among the analytes and intake or animal performance exists due to season, weather, location and many other variables, many of which are unknown. As opposed to empirical equations developed from statistical analysis of relationships among characteristics of samples from a population, theoretical or mechanistic models have been developed (Mertens 1985; Weiss et al. 1992; Van Soest 1994) that theoretically are more robust. However, few such models have enjoyed routine utility by producers or consultants, either due to the complexity of the model or the requirement for numerous inputs.

NIRS to the Rescue

After the early work of Norris et al. (1976), little effort was made to directly estimate *in vivo* forage quality parameters (intake and digestibility) with NIRS, but rather the attention was drawn to estimating chemical composition (crude protein and Van Soest fiber). As the technology was moved to the field to estimate forage quality (e.g., hay quality at auctions), the earlier equations (e.g., Rohweder et al. 1978) were then employed to convert the NIRS estimates of chemical composition to nutritive value and intake. Some of members of the USDA-ARS network, primarily the animal scientists, argued for direct prediction of intake and digestibility using NIRS, not relying on equations developed with limited chemical information (Coleman and Windham 1989). After all, we argued, NIR spectra provide far more chemical and physical information than a few discrete, crude estimates of protein, structural carbohydrate, and perhaps lignin. Furthermore, the paper by Norris et al. (1976) had demonstrated that NIRS could be used to directly predict animal intake and digestibility. Since then, many papers have appeared in the literature (Table 1) demonstrating a relationship of NIRS spectra and forage nutritive value, as well as intake and digestibility determined *in vivo*. Later work demonstrated that direct prediction of digestibility using NIRS was in most cases more precise than the use of chemistry (Table 2). Lippke and Barton (1988) showed that a single, well-chosen wavelength was quite effective for estimating digestible organic matter intake, a combination of intake and digestibility, and highly related to animal productivity (Holmes et al., 1966).

Table 1. Calibration and validation statistics for the direct prediction of *in vivo* forage quality attributes (digestibility and intake) with NIRS spectra of the forage.

			Digestibility, %			Intake, g/kg Metabolic Body Size			
Forage	Statistic	N	Range	R^2	SE	Range	R^2	SE	Reference
Mixed hay[a]	CAL	76	46-77	0.78	3.6	40-114	0.64	8.6	Norris et al.
	VAL	37		--	5.1		--	7.9	(1976)
Mixed hay[a]	CAL	30	52-82	0.67	4.1	96-104	0.71	8.2	Eckman et al.
	VAL	30		0.61	4.8		0.49	10.6	(1983)
Mixed hay[b]	CAL	49	44-67	0.66	2.8	75-129	0.66	7.8	Redshaw et al.
	VAL	17	47-65	0.68	2.4	81-131	0.72	7.6	(1986)
Mixed hay[a]	CAL	45	42-67	0.57	3.3	66-116	0.55	8.4	Redshaw et al.
	VAL	15	47-66	0.47	4.4	62-116	0.83	6.3	(1986)
Grass silage[a]	CAL	101	--	0.81	2.8	--	--	--	Baker & Barnes
	VAL	26	--	0.83	2.1				(1990)
	VAL[c]	38	--	0.83	1.6				
Straw[a]	CAL	81	46-65	0.74	3.3	--	--	--	Givens et al.
	VAL	42		0.65	3.7				(1991)

[a]Fed to sheep.
[b]Fed to cattle.
[c] Independent calibration samples fed at a different facility.

Table 2. Relationship of *in vivo* digestibility by sheep using NIR spectroscopy and various conventional laboratory methods.

		Calibration			Validation					
Forage Type/ Measurement	Method	N	R^2	SEC†	N	R^2	SEP†	Slope	Bias	Reference
Mixed	NIRS†	30	0.67	0.17	30	0.67	0.17	--	--	Eckman et al.
DE†, Mcal/kg	IVDMD†	30	0.59	0.20	30	0.76	0.12	--	--	(1983)
Grass silage	NIRS	122	0.85	25	48	0.76	26	0.93	-0.79	Barber et al.
OMD†, g kg^{-1}	IVOMD†	122	0.74	32	48	0.64	36	0.89	-1.85	(1990)
	PC†	122	0.55	42	48	0.40	47	0.71	2.33	
	ABLIG†	122	0.52	44	48	0.14	53	0.48	1.18	
	MADF†	122	0.34	51	48	0.20	51	0.52	-0.59	
Straw	NIRS	81	0.74	33	42	0.65	37	0.99	-1.24	Givens et al.
OMD, g kg^{-1}	IVOMD	81	0.61	39	42	0.60	40	0.99	-0.90	(1991)
	NDC†	81	0.61	39	42	0.48	49	1.12	-1.24	
	PC†	81	0.60	40	42	0.51	44	0.95	-0.7	

† SEC = standard error of calibration; SEP = standard error of prediction; NIRS = near-infrared reflectance spectroscopy; DE = *in vivo* energy digestible; IVDMD = *in vitro* dry matter digestibility; OMD = *in vivo* organic matter digestibility; IVOMD = *in vitro* organic matter digestibility; PC = pepsin-cellulase; ABLIG = acetyl-bromide lignin; MADF = modified acid detergent fiber; and NDC = neutral detergent followed by cellulase digestion.

Intake is more difficult both to measure and to predict than is digestibility, largely because intrinsic properties of the feed only partially explain variability in intake (Heaney et al. 1966; Heaney 1970; Coleman and Windham 1989). However, several reports showed that the residual SE from NIRS equations approximated 10% of the mean of actual intake, and are similar to other published results using chemistry or chewing behavior (e.g., Ward et al. 1982). Perhaps the most successful, and most likely to gain practical acceptance for a targeted audience, is that reported by Barber et al. (1990) and Baker and Barnes (1990), in which a great number of grass silages were collected from producers and farmers throughout the UK and fed to lambs under a rigid set of conditions to obtain *in vivo* digestibility. The decision to evaluate a single entity (grass silage) and control the experimental protocol provided excellent relationships between NIRS spectra and digestibility. Because all animals were fed at a restricted level of maintenance, intake could not be estimated. However, this provided a very repeatable measure of digestibility from which to calibrate NIRS.

The problem with directly predicting *in vivo* measurements (intake and digestibility) with routine chemistry or NIRS has been in obtaining sufficient numbers of samples for which reference data were obtained under carefully controlled and defined conditions. A further problem is that of monitoring the equations to ensure that new samples do indeed fit the spectral matrix of the calibration data set. In this regard, it is easier to use published results in which intake and diet quality were reported along with nutritive composition of the feed source (Moore et al. 2007) than NIRS, because NIRS spectra normally are not tabulated in the scientific literature. Even with these limitations, reports in the literature began to emerge with NIRS equations for direct prediction of intake and digestibility based on spectral characteristics of the feed (Table 1).

How Did Feces Get Into the Picture?

In 1980, Dr. Woody Barton, an ARS chemist from Athens, Georgia came to our facility in El Reno, Oklahoma for one year. During that time, I learned the basics of NIR spectroscopy. However, still being an animal nutritionist, we were conducting *in vivo* intake and digestion trials with the intent of developing relationships between chemical and physical properties and attributes of forage quality. He convinced me that the NIR spectrum contained much more information than we were analyzing with Kjeldahl protein and Van Soest fiber. We began to investigate how to merge the spectra of hay fed and feces voided from a single animal to determine 'spectral digestibility.' We envisioned calculating digestibility coefficients at each 2 nm wavelength. The first attempt was to attach the fecal spectrum at the end of the feed spectrum (Figure 1), which did not lend itself to subtraction and division within the spectra with our current software. Therefore, 'spectral digestibility' came much later (Coleman and Murray 1993), but with the feed-fecal spectrum in one record, we were able to determine the relationship of intake and digestibility to both feed and feces. Not surprising in retrospect, the multiple wavelength selection program (stepwise regression) selected wavelengths from both the feed and feces to predict digestibility. However, only wavelengths from fecal spectra were chosen for predicting intake.

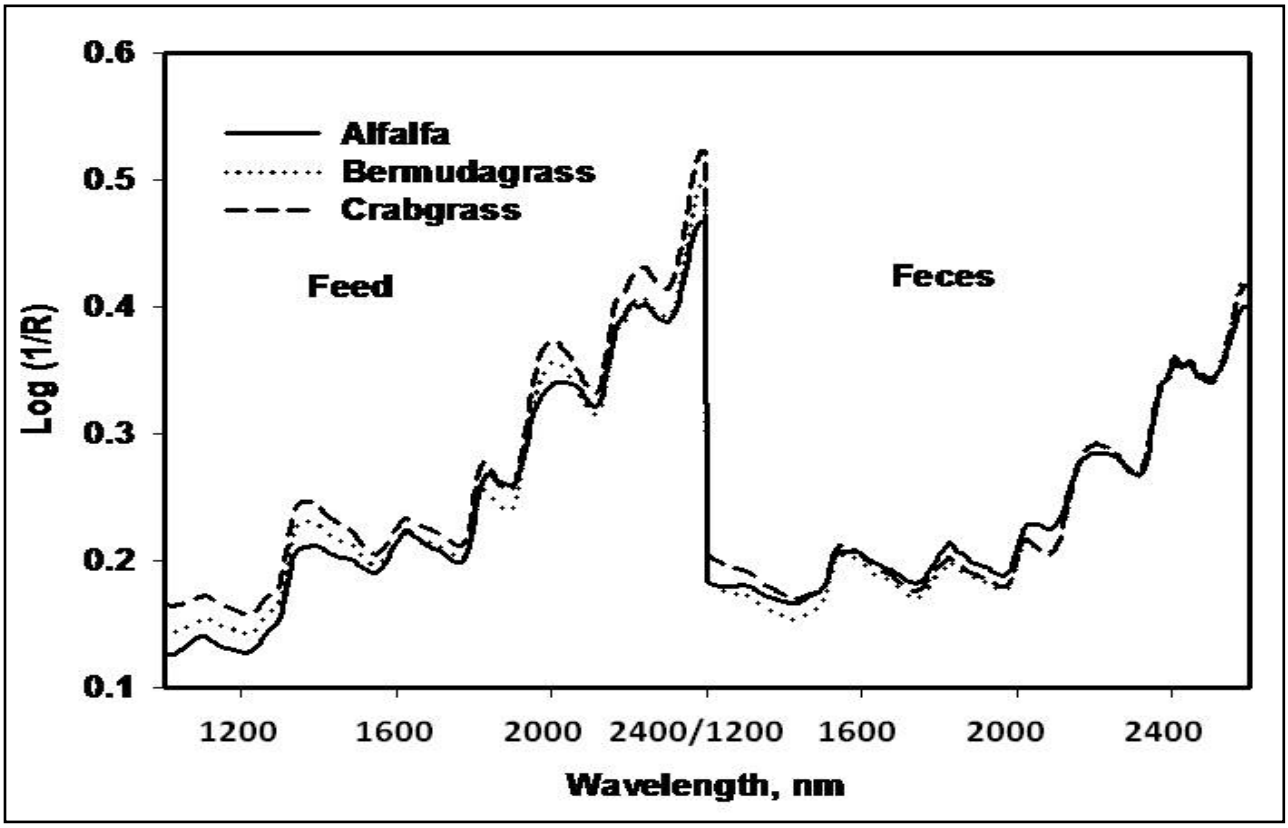


Figure 1. Spectra of hay and the feces from animals eating the hay.

Fecal Indices to Estimate Intake and Diet Quality

While standard laboratory or NIRS may be used to assay chemical and nutrient composition of forage available, under grazing it is quite difficult if not impossible to obtain a sample of the diet consumed by the animals. To address this problem, simultaneously in the 1980s, Dr. Bill Holloway in Tennessee and Dr. Jerry Stuth in Texas were investigating techniques to determine intake and diet quality of grazing animals with the intent to extrapolate to large numbers of animals or to commercial producers. Both were attempting to use a fecal index to estimate intake and nutritive attributes of the diet (Holloway et al. 1981; Leite and Stuth 1990). In a conversation with Dr. Holloway, I related how spectra of feces appeared to be more closely related to intake than spectra of hay. Being interested in pursuing the potential of NIRS for a fecal index, he provided a group of fecal samples (Holloway et al. 1981) on which he had measured intake (stall fed) and digestibility (marker). The results were promising (Table 3), but quite modest when compared to our experience with forage chemistry and even *in vitro* digestibility. However, Dr. Holloway was quite impressed because when compared to discrete chemistry, the NIRS index was more closely related to intake and diet nutritive value. In 1985, Dr. Jerry Stuth and I were traveling on a school bus in China and discussing the difficulties of estimating intake and diet quality of grazing animals. When I mentioned the preliminary results that we had with the Holloway data, he was interested in collaborating, using fecal samples on which he had estimated diet quality and intake using markers with grazing cattle (Olson 1984; Table 3) to determine if NIRS had potential for his application. The results again were promising. The feces for these evaluations were generated in a grazing system in which the paddock was greatly overstocked for a short period of time. Esophageal and fecal samples were collected during a 7- to 10-day period and composited for analysis. Difficulties arose because what the animal ate on day 1 was quite different than on day 3 or 4 because of the heavy grazing pressure. Therefore, agreement between laboratory reference values obtained on diet samples (esophageal) and fecal was faulted, not because of lack of agreement in methods, but because feces taken on a given day were not representative of the diet selected on that day. A delayed sampling for feces may have helped, but without rate of passage, the length of delay was only conjecture. A different sampling scheme was suggested in which the paddock was grazed to a certain level and then all animals except a few testers were removed so that change was not so rapid. Since then, several reports have demonstrated excellent results for estimating both intake and digestibility using NIRS spectra of feces (Table 3). The results from Boval et al. (2004) as well as when average values were used, Coates (2005) for calibration are of particular interest because in these studies, the residual SE are smaller than normally reported for differences among animals fed the same diet. Of importance is the use of diet average for the reference data. It remains to be determined if this technique can be used to determine intake of individual animals, but David Coates (personal communication) suggested the answer is no because the predicted intake of two animals fed the same forage were very similar but their actual intake was largely different (see also Chapter 3 this volume).

While intake and in vivo digestibility of the diet are more intriguing because of the difficulty and cost of direct (if there is such a thing) determinations, analysis of the feces

can provide other attributes of the diet. Of these, crude protein has been predicted quite readily (Lyons and Stuth 1992; Coates 2005) and its incorporation into the fecal-NIRS:NUTBAL model has been beneficial toward timing protein supplementation under a wide variety of grazing situations.

Table 3. Relationship (calibration) of *in vivo* digestibility and intake and near-infrared reflectance (NIR) spectra of feces[a].

Forage Species/ Type	Animal Species	N[b]	Digestibility, % Range	RSE[b]	R^{2b}	Intake, g/kg BW Range	RSE[b]	R^{2b}	Data Source
Fresh C_3 herbage	Cattle[d]	37	35-80	3.7	0.88	7.7-21.4	1.7	0.81	Holloway et al. (1981)
Native range, C_4	Sheep	136	38-71	3.1	0.82	7.7-44.7	3.5	0.75	Olson (1984)
Ryegrass pasture	Cattle[d]	59	--	--	--	9.1-31.0	3.4	0.82	Flinn et al. (1992)
Bluestem pasture	Shp/Cat[f]	96	59-70	1.3	0.73	12.6-39.4	4.4	0.60	Forbes and Coleman (1993)
Fresh C_4 herbage	Cattle[e]	87	53-74	2.0	0.69	15.2-23.4	1.3	0.52	Boval et al. (2004)
Mixed tropical	Cattle[f]	--	31-85	3.9	0.80	3.3-30.4	2.2	0.79	Coates (2005)
Mixed tropical	Cattle[c]	--	42-72	1.7	0.95	4.2-28.6	1.7	0.85	Coates (2005)
Fresh C_3 herbage	Cattle[g]	86	30-83	5.4	0.58	7.8-32.4	2.9	0.66	Holloway (Unpublished)
Mixed hay		33	46-75	2.5	0.86	12.5-27.1	3.2	0.31	Coleman (Unpublished)

[a]All intake units based on g DM/kg body weight/d.
[b]N = number of individual determinations of intake used for calibration; RSE = residual standard error; R^2 = coefficient of determination.
[c]Digestibility determined by Cr_2O_3 marker and intake directly.
[d]Digestibility was determined on 75 diets with a total of 295 fecal spectra and intake was determined on 117 diets with 472 fecal spectra. Reference data was that for individual animals.
[e]Digestibility was determined on 78 diets with a total of 313 fecal spectra and intake was determined on 117 diets with 472 fecal spectra. Reference data was averaged for each diet.
[f]Digestibility determined with sheep and intake by cattle.
[g]Digestiblity determined by *in vitro* fermentation of diet sample and intake by fecal output estimated by markers.

This early work demonstrated how diversity of sampling conditions (weather, location, forage species, season, etc.) influence the spectral matrix of feces that can be described by principal component (PC) analysis (Figure 2). In this case, a large number of samples from the Uvalde population form a core 'neighborhood' (0.065-0.085 PC1 x -.005-0.005 PC2) that is sample dense. A small number of Uvalde, Texas samples (probably a different sampling date) are spread across the positive end of PC2. The La Copita and College Station, Texas populations are largely disjunct from the core neighborhood. Predicted values from the La Copita and College Station populations would have a high probability of being consistently higher

or lower than the reference values (systematic bias) because of the matrix diversity. However, this is not always the case, and it has been rather difficult to prove that neighborhood outliers cannot be predicted by an equation, nor that samples well within a neighborhood will be predicted correctly. Spectral matrix refers to spectral characteristics of feces (or forage, for that matter) that either have no relationship to the chemical, nutritional, or other factors of interest (intake and diet quality), or else the matrix differences interfere with their estimates (Coleman et al. 1989; 1995). The Forage Task Force had already recognized the issues of matrix, and two theoretical approaches were devised to overcome them:

1) Structure the data set very tightly (same species, grind, drying method, growing conditions, etc.) and develop an equation that is very effective for samples similar to the ones in the calibration data set. This technique was very good for 'double-sampling' techniques in which a proportion of the total sample set (e.g., forage breeder's nursery) was used to develop equations and the remainder predicted from the equations.

2) Develop a broadly comprised data set with samples from as many diverse situations (species, time, locations, etc. as possible). These equations would be more robust, but prediction of values on a single sample would likely be more variable (greater chance to differ from the reference data). However, the prediction would likely be more accurate than if predicted with a tightly structured data set that did not include samples like the ones being predicted.

The 'broad equation' approach (#2 above) would be more desirable for estimating producer samples and others in which the matrix is unknown or uncontrollable. Regardless, a major difficulty with estimating *in vivo* quality attributes in a predictive mode, whether feed or feces is analyzed, is the difficulty in monitoring the equation for unknown samples with different matrices (those collected in different spatial and temporal conditions). The techniques for obtaining diet quality and intake have already been noted as being quite laborious and costly (Coleman et al. 1989). Furthermore, sufficient quantity of the feed would not be available for *in vivo* determination (feeding an animal) on most samples.

For grazing herbivores, feces integrates the processes of selectivity, intake level, mastication, rumination, and all the other processes that are difficult to quantify but are contributors to estimates of forage quality. Therefore, because some of these processes are difficult or impossible to measure, feces should provide a better indicator of *in vivo* diet quality under grazing conditions than analysis of a clipped forage sample. Provided there is a relationship between the spectra of feces and diet quality, then feces is the preferred medium for analysis under grazing conditions, largely because it solves the problems associated with selectivity by the animal and sampling the diet. Another advantage of NIRS analysis of feces is that many dietary attributes (e.g., crude protein) can be estimated from a single sample that is easily obtained. Therefore, fecal NIRS (F.NIRS) offers a rapid analytical technique to assist in assessing supplementation needs for grazing animals.

From our first collaboration with Dr. Stuth, he redesigned the grazing trials to provide better agreement between fecal and diet samples. Lyons and Stuth (1992) reported excellent calibrations for estimating

diet crude protein and digestibility of grazing animals using NIRS. The incorporation of predicted data from fecal analysis using NIRS into the NUTBAL model has developed into a broad-based decision support system (Stuth 1997) and is being used world-wide. Monitoring of equations for accuracy and for samples to 'fit' the calibration matrix in diverse situations remains a difficult issue. Moore et al. (2007) suggested criteria for evaluating equations based on the inherent variability observed in the reference data, not based on goodness of fit between predictors and reference data. They suggested that differences between predicted and observed measures of digestibility be within 5% of the mean of the samples in the test data set, whereas for intake, the differences should be within 10% of the mean. This corresponds to observations in the literature that variability among animals when measuring digestibility and intake are 5 and 10%, respectively.

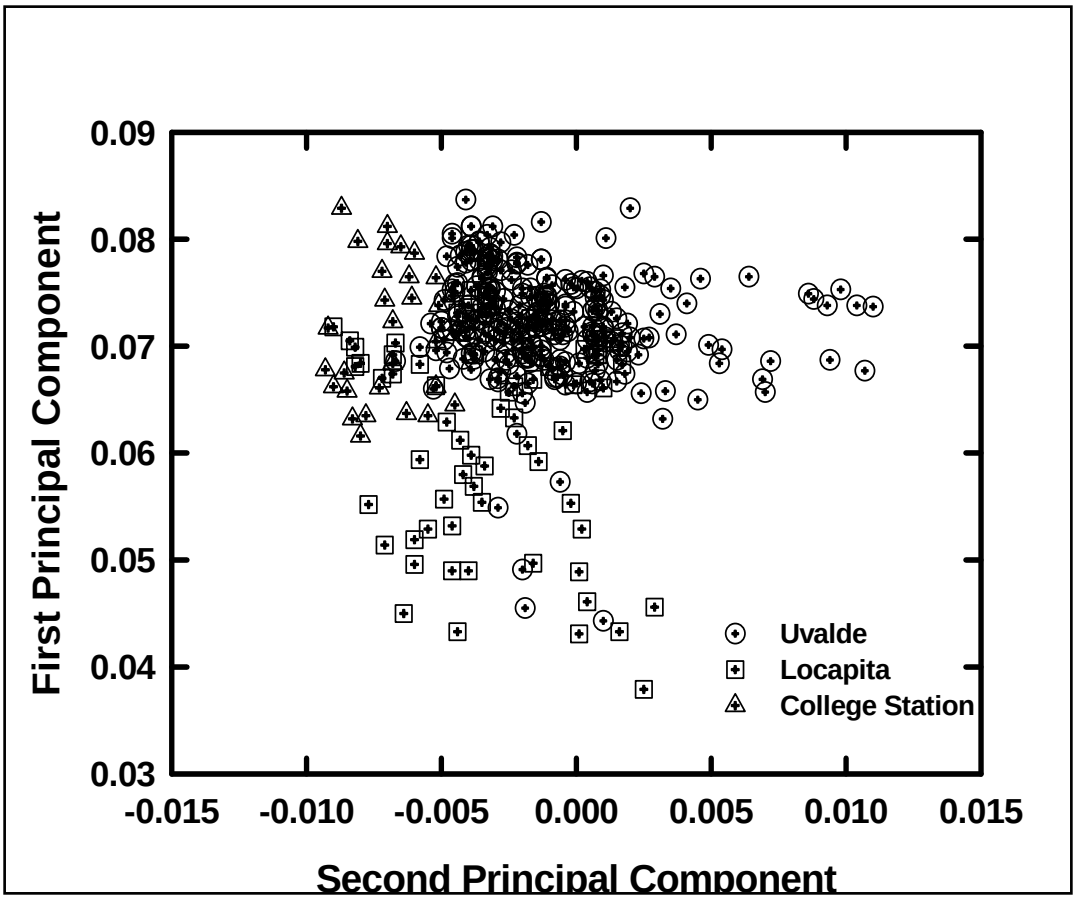


Figure 2. Scatter diagram of the first and second principal component of fecal spectra from cattle grazing in three different locations in Texas (Coleman et al. 1995).

The use of F.NIRS for estimating intake has not been adopted as readily as that for diet quality even though early results indicated intake was as closely related to fecal NIR spectra as was digestibility (see Table 3) when intake was measured directly. However, often the methods used in obtaining measures of direct intake (forage fed in a manger and refusals weighed back) cause bias (lower intake) due to the feeding

conditions. Intake estimated from pasture studies using markers may be both biased and have a high degree of variability due to the methods (Coleman 2006). Therefore one reason F.NIRS has not been readily adopted appears to be a reluctance by clientele (producers, consultants, advisors) to accept values for which marginal statistical relationships exist because they were not well informed on the native variability in measurement of intake and who expected a much tighter relationship than was possible. Early use of NIRS for direct forage analysis suffered the same reluctance because the number from NIRS did not exactly match the number obtained from the reference laboratory, even though two assays by the reference method did not agree either.

What's Ahead?

Miniaturization and portability of instruments are already shaping the next generation of the use of NIRS for pasture management. Recently Phillips et al. (2007) compared recommendations for supplementing grazing steers using three methods. The control was the 'Oklahoma Gold' program that had been in use for many years and was based on beginning supplementation at a certain time. The second method consisted of assessing diet quality by fecal NIRS-NUTBAL. The third method employed a portable spectrometer calibrated to provide estimates of crude protein of standing forage on offer. In terms of rate of gain the F.NIRS and the portable NIR were superior to the standard 'Oklahoma Gold' recommendation, which is based on time. Additional advantages with fecal analysis compared to direct measurement of CP in the standing crop include removal of the problem of diet sampling. Furthermore, fecal characteristics should provide greater information on the selection, biting, mastication, digestion and excretion than feed. After all, feces is an integrator of eating, digestion, and absorption of nutrients and lends itself well to the technology we have in NIRS. Further advances include combinations of near-canopy and satellite based remote sensing of standing herbage for both quantity and quality analysis. Analysis of feces by NIRS has been used to predict botanical composition of diets (Walker et al. 2007) and physiological status (pregnant or not, sex) of grazing animals (Tolleson et al. 2005). Since F.NIRS is an indirect, predictive technology, care must be exercised in population development and maintenance. Sophisticated statistics do not preclude the need for sound reasoning when combining populations and predicting unknown samples that are different from the population on which the equation is based. We still have a lot to learn concerning when an equation is performing under control (Shenk et al. 1989).

SUMMARY and CONCLUSIONS

Near-infrared spectroscopy, and especially diffuse reflectance, has provided a tool for feed analysis that is becoming routine in use. Being a predictive method, accuracy as well as precision, is dependent on the quality of the reference data, breadth of the sample set used for calibration, and methods for monitoring calibration accuracy. However, the stability of instruments, and more recently their portability make this an exciting area for development. Collaboration through the exchange of databases is a necessity to amass sufficient data sets on which to base valid calibrations and the breadth of matrix conditions to make the equations robust.

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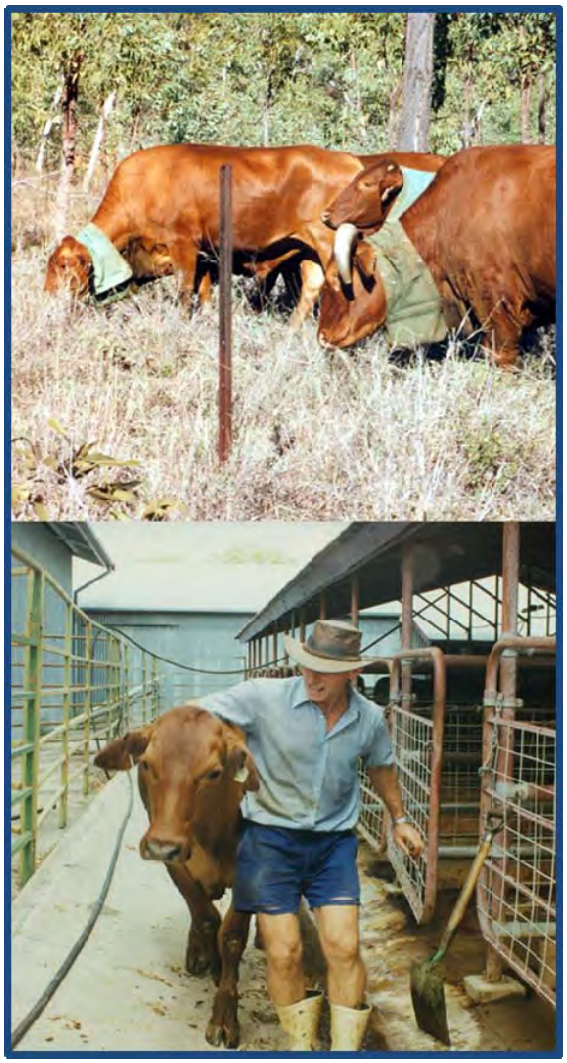
Chapter 3. Fecal NIRS Calibration for Predicting Protein and Digestibility in the Diet of Cattle: Fistulate and Pen Feeding Procedures for Generating Diet-Fecal Pairs

D. B. Coates and R. M. Dixon

Objectives: To describe different ways to create diet-fecal pairs for predicting the nutrient composition of free-grazing cattle diets and to discuss the pros and cons of each method and how combining them can create good calibration equations.



Key Points

- Diet-fecal pairs are diet samples from which nutrient values were obtained and fecal samples from which spectral data are collected. Three different procedures were used: 1) esophogeal fistulated diets and feces from intact resident animals (EF); 2) animals fed hay in confinement (PENHAY); and 3) animals fed fresh-cut forages in confinement (PENFRESH). Diets were analyzed for dry matter digestibility and crude protein (CP).
- Based on CP, the EF method predicted the PENHAY and PENFRESH methods best. But there were still significant differences between predicted and actual values.
- Validation of dry matter digestibility among the three methods was poor.
- Combining the data from the three methods created substantially better calibrations.
- Pros and cons of all three methods are described in this chapter, and combining data from all three methods will be required to develop useful calibrations.

INTRODUCTION

Use of fecal Near Infrared Reflectance Spectroscopy (F.NIRS) for measuring dietary attributes such as crude protein concentration and DM (dry matter) digestibility differs from most NIRS applications in several respects. Importantly, in developing calibration equations, constituent reference values and spectra are measured on different substrates: constituent values are determined by analyzing diet samples using appropriate laboratory techniques while the NIR spectra are obtained by scanning fecal samples from animals consuming the same diets. Thus the term "diet-fecal pairs" is often used to describe these samples for developing calibration equations, which are defined as derivative calibrations in Chapter 1. Obviously an important characteristic of diet-fecal pairs is that the sample analyzed for diet reference values must be truly representative of the diet consumed and the sampled feces also must be properly matched with that same diet.

A second unusual aspect of F.NIRS technology is that the dietary material of interest is modified by digestion in the gastrointestinal tract before the spectra are measured. Therefore, there is an implicit assumption that there are stable and close correlations between the spectral absorbances of feces at certain wavelengths and the dietary attributes of interest. It appears that deviations from such correlations between diet and fecal components introduce errors with some types of diets.

In this paper we discuss the development of F.NIRS technology for cattle production in the extensive grazing systems of northern Australia. We focus on the techniques and methods used for obtaining diet-fecal pairs that meet the requirements of robust calibration equations.

The Approach Adopted

There are a number of conditions that generally are accepted as being necessary for developing robust NIRS calibration equations to quantitatively measure constituents of feeds and forages, and these have been described in various reviews (eg., Shenk and Westerhaus 1993; Williams 2001; Westerhaus et al. 2004). These conditions generally also apply to development of calibration equations to measure diet attributes from fecal spectra. Although there are differences in the approach adopted by various research groups, essential conditions include:

- Samples in the calibration set should, as far as possible, encompass the full range of constituent values likely to be encountered in the open population for which the calibration equation is to be used.

- Samples in the calibration set should also encompass the full spectral diversity of samples likely to be encountered in the open population. This requirement relates especially to spectral diversity unrelated to the specific constituent being determined and includes spectral diversity arising from such influences as plant species, stage of growth, soil type, climate and weather, and non-specific year effects (and in the case of F.NIRS, animal effects).

- Errors in laboratory reference values must be minimized. It has been argued that the accuracy of calibration equations can only be as good as the accuracy of the laboratory reference values. Although there are situations where NIRS analysis can improve upon the accuracy of reference values (DiFoggio 1995; Coates 2002), it is nevertheless extremely important to

minimize both the sampling errors and the analytical errors associated with the reference values.

A further consideration for the development of F.NIRS calibrations to measure diet quality of grazing cattle is that the acquisition of diet-fecal pairs is usually laborious and costly in experimental resources. Thus it is particularly important to ensure that the methods will, as far as possible, meet critical criteria such as those described above, while recognizing that the experimental resources available are not likely to be sufficient to encompass all the situations to be encountered; in our case, across the vast array of systems in the northern Australian grazing industry.

The development of F.NIRS technology in Australia began in the early 1990s soon after the publication of the research of Lyons and Stuth (1992) in Texas, US, which demonstrated that it was possible to use this technology to measure the crude protein (CP) concentration and digestible organic matter in the diets of free grazing cattle. Developmental work was conducted in two CSIRO (Commonwealth Scientific and Industrial Research Organisation) research projects with funding support from Meat and Livestock Australia (formerly Meat Research Corporation). Each project was of three years duration, the first during 1995 to 1998 (Coates 1998) and the second, which also involved Australian State Departments of Agriculture and universities, during 2001 to 2003 (Coates 2004). This information has been reported in part by Dixon and Coates (2005); Coates and Dixon (2007); Dixon et al. (2007). Three different methods were used in these studies to generate diet-fecal pairs in the development of calibration equations, and this chapter investigates the advantages and disadvantages of each method.

Methods for Creating Diet-Fecal Pairs

A principal difficulty in generating reliable diet-fecal pairs is to obtain a sample of the diet that is truly representative of the diet ingested by the cattle from which the feces are collected. As explained above, samples of the diet are needed for laboratory analysis, usually using conventional wet chemistry procedures, to generate the reference values. Clearly there will be errors in the reference values assigned to fecal spectra if the forage sample analyzed is not truly representative of the actual diet. This problem can be minimized by feeding cattle in pens where the opportunity for selection can be almost eliminated and where the forage offered and the forage refused can be sampled directly to measure the diet ingested. The problem is more difficult to address in grazed pastures, particularly tropical pastures, where the botanical and chemical composition of selected forage often bears little relationship to the composition of the pasture on offer. The approach usually adopted to measure the diet of grazing cattle or sheep is to use esophageal fistulated (EF) animals to obtain samples of grazed forage that are referred to as extrusa, which represent the selected diet. This approach is used commonly in diet selection studies and was used by Lyons and Stuth (1992) in their pioneering research. We used three methods involving either pen-fed cattle or grazed pastures coupled with EF sampling to generate diet-fecal pairs.

Esophageal Fistula Method. During the first project, most of the effort was directed to generating diet-fecal pairs for cattle grazing pastures in small paddocks within existing grazing trials and using the EF diet sampling procedure (Coates 1998). Paddocks were chosen for uniformity of soil type and pasture within the paddock and also for sufficient area to support resident herds of 3 to 5 head of growing cattle

(resident cattle). A range of pasture species and mixes were represented at sites in northeast Queensland, and included native pastures, sown grass pastures, and sown grass/legume pastures. On the day of sampling, a single fecal sample was collected from each of the resident cattle, and these samples were scanned to provide individual spectra. Plant material representing the diet was obtained by sampling with 3 to 6 EF steers that were familiar with the pastures and paddocks. Laboratory analysis was conducted on individual samples of extrusa, and diet reference values were calculated for pairing with each spectrum. This procedure is described below under the heading "Laboratory procedures used to measure diet-fecal pairs." In these trials the resident cattle were usually not the EF animals and they had not been surgically modified. Sampling in this way was conducted over the three years of the project and at four different locations, Lansdown, Cardigan, Hillgrove, and Springmount (Figure 1). The locations differed substantially in soil type and climatic factors, but the maximum distance between locations was about 450 km. The set of diet-fecal pairs generated in this way contained 115 different diets and was designated the EF method set. Diet-fecal pairs representing an additional 30 grazed diets from Brian Pastures were added to the EF set during the second project.

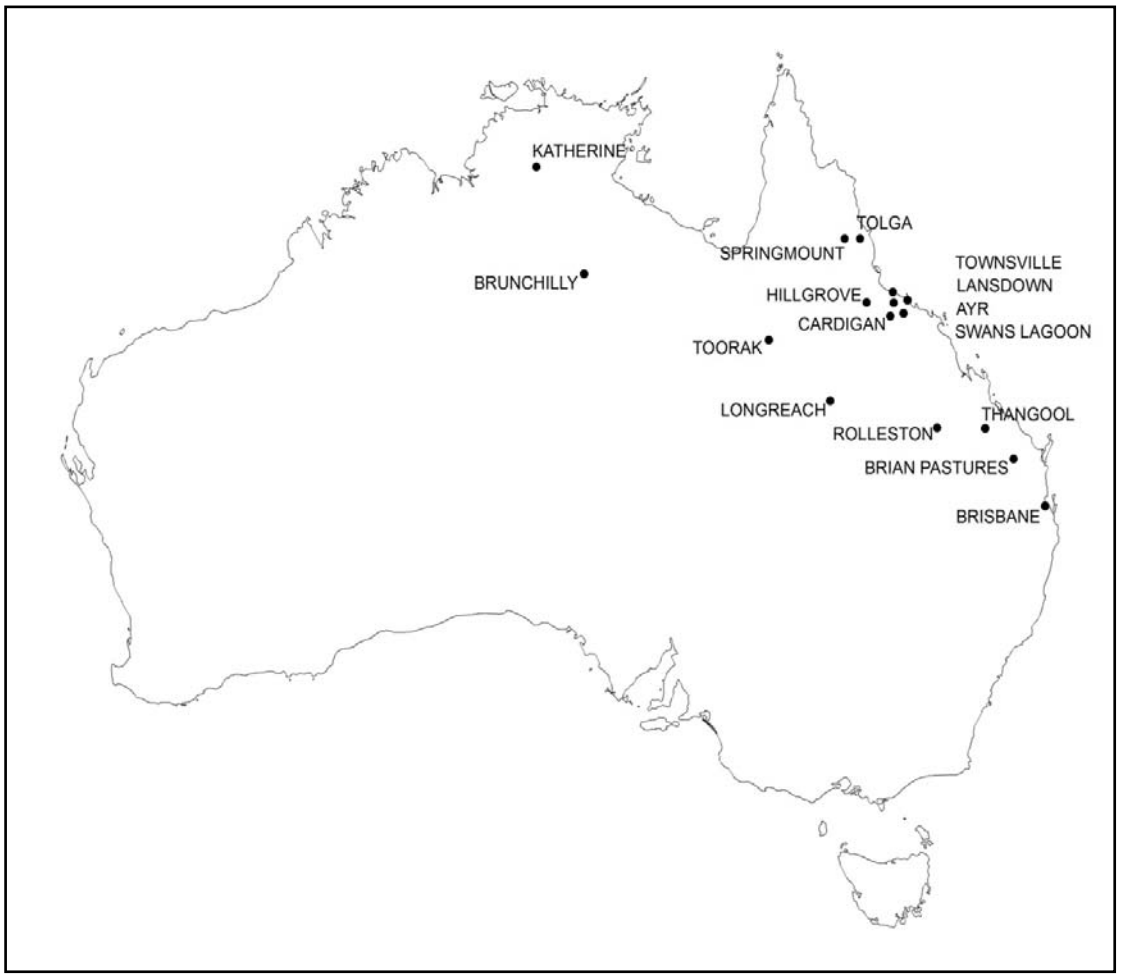


Figure 1. Forage sources for generating diet-fecal pairs. EF sampling sites: Lansdown, Cardigan, Hillgrove, Springmount and Brian Pastures. Hay origins: Katherine, Tolga, Townsville, Lansdown, Ayr, Swans Lagoon, Toorak, Longreach, Rolleston, Thangool, Brian Pastures and Brisbane and other (unknown) sites. PENFRESH feed sources: Katherine, Brunchilly, Townsville, Lansdown, Swans Lagoon and Brisbane.

PENHAY Method. Another set of diet-fecal pairs was generated in conventional live animal digestibility trials where individually penned cattle were fed chopped, sun-cured hays at about 10% above voluntary intake. In these trials, the adaptation period was 9 days followed by 8 days of total fecal collection. A total of 78 different forage diets of C_4 grass hays, some C_3 temperate grass hays, legume hays, and mixtures were fed in these trials (Coates 1998). Hays were sourced from various locations to diversify the geographic spread of the forage sources. Animal replicates per diet varied from 3 to 10. Another 15 hay diets were fed to cattle in pens, but the duration was insufficient to measure digestibility. Diet-fecal pairs from all 93 diets were designated the PENHAY set.

Subsamples of the diet offered were taken at morning and afternoon meals for the final eight days of live animal digestibility trials. Forage subsamples were bulked within diet, and the composite sample was processed and analyzed to determine the relevant reference values. Forage refusals were collected so that constituent values for forage consumed could be calculated. We established to our satisfaction that such adjustments had negligible effect on the calculated reference values. Therefore, in the later trials, the constituent values for the forage offered were used as the reference values in diet-fecal pairs. Fecal samples for diet-fecal pairs were collected on the final 2-3 days of the feeding period and bulked within animals before obtaining the necessary spectra.

PENFRESH Method. Feeding trials during the second research project, 2001 to 2003, were mainly short duration trials where penned cattle were fed forage harvested directly from the paddock (Coates 2004). Areas of various pasture types as uniform as possible for plant species and maturity were selected, and the feed usually was harvested using a tractor-mounted flail-type forage harvester. In a few instances where conditions were appropriate, the forage was harvested using a domestic lawn mower, whereas in others, such as where a browse species like leucaena (*Leucaena leucocephala)* or mulga (*Acacia aneura*) was a dietary component, part or all of the diet was hand harvested. During the pasture growing season when the forage was green, pasture was harvested either before each meal (morning and afternoon) or once a day, whereas pasture which had matured and aged was harvested once a day or less frequently. Trials of this kind were conducted at five geographically dispersed sites: Lansdown Research Station near Townsville, Swans Lagoon Research Station near Ayr; Mt. Cotton Research Farm near Brisbane; Katherine Research Station near Katherine in the Northern Territory, and Brunchilly Station on the Barkly Tableland in the Northern Territory (Fig.1). Feeding continued for 5-10 days, the main requirement being for the feces to equilibrate with the diet being fed. This interval was tested in the first of such trials conducted at Lansdown by monitoring F.NIRS predictions of diet quality using existing equations (Fig. 2). Five days was sufficient for feces to equilibrate with the new diet. In most of these trials, cattle grazed similar pasture to that being harvested before entering the pens. Diet-fecal pairs from the 77 different diets fed were designated the PENFRESH set.

Subsamples of the diet offered were taken at morning and afternoon meals for the whole feeding period for PENFRESH diets. Subsamples for each meal were analyzed separately. Fecal samples were collected daily, and each sample was analyzed separately to determine when fecal

spectra had stabilized in relation to the diet being fed. Only those spectra obtained after equilibrium had been reached were used for diet-fecal pairs.

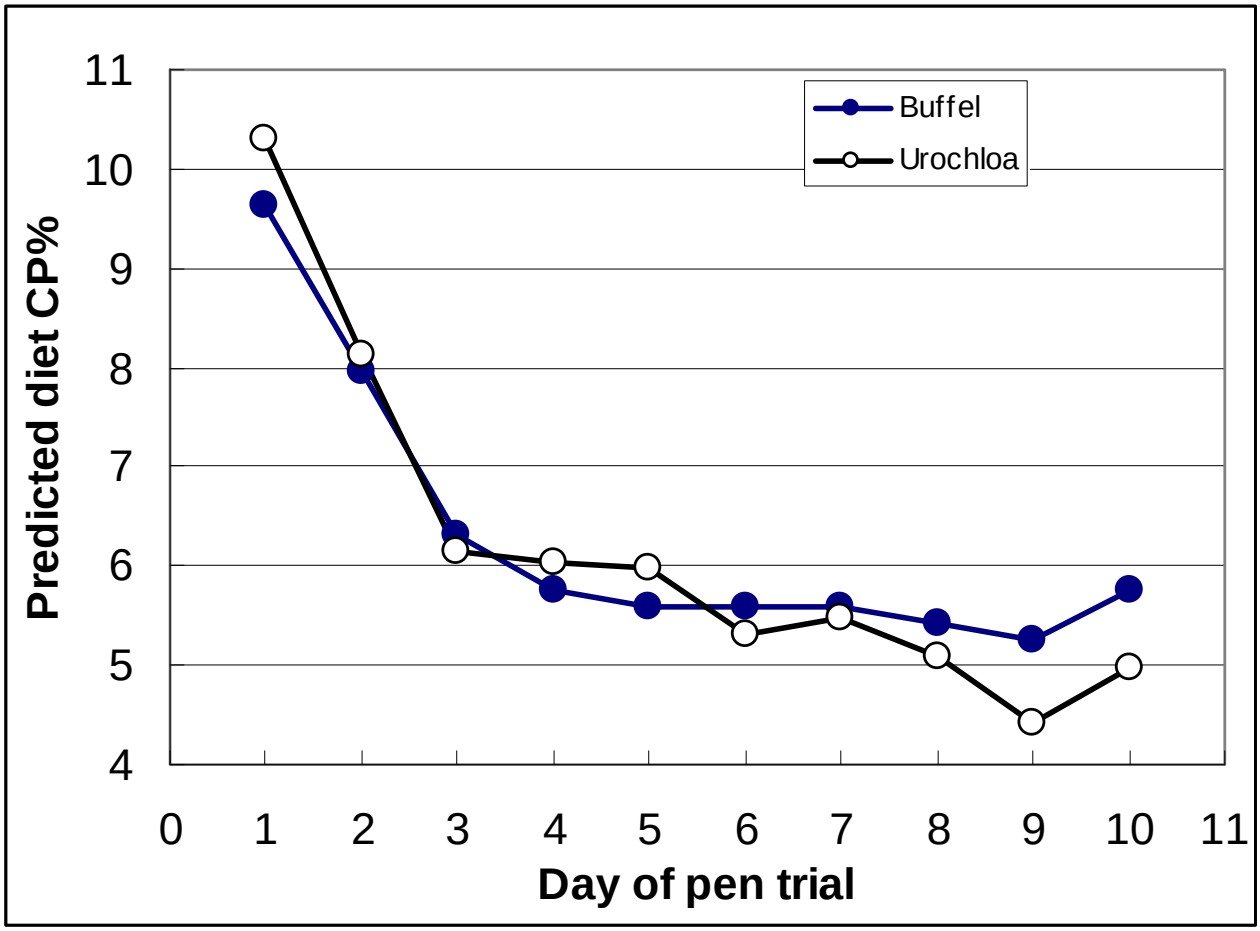


Figure 2. Changes in F.NIRS predicted diet CP% after cattle entered pens and were fed freshly harvested green buffel grass or Urochloa. The trend for a continuing slow decline in predicted diet CP in the Urochloa diet from day 5 probably reflected a progressive slow decline in the protein concentration of the harvested grass with increasing maturity.

Measurement of Diet Nutrient Composition and Fecal Spectra

Samples of the diet, including extrusa from EF steers, were analyzed for total CP (N x 6.25) and for estimated dry matter digestibility (DMD) using the pepsin-cellulase two-stage artificial rumen digestion method of McLeod and Minson (1978). An in-house regression developed from 54 forage diets of known live animal DMD was used to calculate estimated live animal DMD from artificial rumen DM disappearance. Dry matter digestibility of EF samples also was adjusted for the effect of salivary contamination of forages on artificial rumen DM disappearance (Coates and Mayer 2009).

A potentially serious problem with the EF method, at least for tropical pastures, is that samples of extrusa collected from EF cattle do not provide reliable estimates of the botanical and chemical composition of diets selected by resident cattle (Coates et al. 1987; Carulla *et al*. 1991; Jones and Lascano 1992; Coates 1999). The problem exists

even when the EF cattle are also the resident cattle (Clements et al. 1996). If collected extrusa does not represent accurately the integrated diet of the resident cattle (hereafter simply referred to as the integrated diet), then analysis of extrusa will not provide accurate reference values for diet-fecal pairs. We term such errors "mismatch" errors. In tropical pastures, mismatch errors in constituents of interest are often associated with differences in the chemical composition of the grass (C_4) and non-grass (C_3) components, especially in grass-legume pastures. For diet CP, however, we adopted the procedure described by Coates (1999) which uses $\delta^{13}C$ (the ratio of the stable isotopes ^{13}C:^{12}C) for improving the reliability of the reference values by correcting extrusa CP concentrations for differences between the C_3/C_4 proportions of extrusa and the integrated diet using measurements on both extrusa and feces. The correction is specific to tropical pastures and is based on the linear relationship between the CP concentration of a forage mixture and the proportion of C_3 plant material in the mixture. The corrections were considered to be of critical importance in reducing mismatch errors in the reference values because many of the grazed pastures were grass/legume (C_4/C_3) mixtures. In contrast to CP concentrations, we were unable to correct for mismatch errors in DMD reference values of EF diet-fecal pairs (see Coates 1999).

Fecal samples were dried (65^{o}C) and then ground through a 1 mm screen using a FOSS Tecator Cyclotec Laboratory Mill. The processed samples were scanned in a FOSS NIRSystems 6500 spectrometer fitted with a spinning sample cup module. All calibration equations referred to in this paper were developed using ISI software (Infrasoft International) on first derivative spectra with multiplicative scatter correction (SNV and detrend), wavelength bandwidth of 700-2500 nm, and modified partial least squares (MPLS) regression.

RESULTS

Relation to Method

Calibration. To compare the efficacy of the three methods, separate calibration equations were developed for each set of samples (EF, PENHAY, and PENFRESH) and also for the three sets combined (COMBINE; Table 1). Each calibration equation was then used to predict diet CP and DMD on the other sets and the prediction statistics computed (Table 2).

Although all calibration equations were satisfactory as judged by SEC, SECV and R^2 values, there was substantial variation in SE values (SEC and SECV) among the different calibrations particularly with respect to diet CP. However, meaningful comparisons between the diet-fecal pair methods were difficult because of the differing composition of the calibration sets which varied with regard to sample number, number of different diets, range of constituent values, pasture species represented and geographical diversity. Although the PENFRESH calibration equation had the lowest SE values for both diet CP and DMD, this was associated with a smaller range in constituent values (for CP) and a less diverse sample set with respect to pasture species, locations and years represented. Conversely, although SE values for diet CP in the PENHAY calibration were the largest, the PENHAY sample set also had the largest range in diet CP, the greatest diversity in pasture species represented, including a number of C_3 grasses as well as many C_4 grasses, and the greatest geographical diversity in relation to the origin of the different forages.

The SE values for diet CP in the EF calibration were highly satisfactory in view of the potential problems associated with diet sampling using EF cattle, particularly problems that can arise due to mismatch errors as described previously. We conclude that the correction procedure used to reduce mismatch errors in CP reference values was probably effective though it was apparent that the incidence of outliers was much higher for the EF calibration than for the PENHAY and PENFRESH calibrations. In contrast to CP, SE values for the EF and COMBINE calibrations for DMD were substantially higher than those for the PENHAY and PENFRESH calibrations. It is probable that mismatch errors in DMD reference values within the EF sample set adversely affected the calibration statistics for both the EF and COMBINE sample sets (Table 1) and also the validation statistics presented in Table 2.

Table 1. Calibration statistics for diet crude protein (CP) and dry matter digestibility (DMD) according to method (EF, PENHAY, PENFRESH) and of the combined sets (COMBINE).

Method set	Calibration Equation	N[a]	Constituent Range (SD)	Outliers[b] eliminated	Number Terms	SEC	SECV	R^2
			Dietary crude protein, %					
EF	EF	551	3.0 – 19.7 (3.24)	24	9	0.81	0.83	0.94
PENHAY	PENHAY	393	1.9 – 25.4 (5.03)	2	9	1.03	1.08	0.96
PENFRESH	PENFRESH	256	1.5 – 13.9 (2.28)	3	10	0.53	0.56	0.95
COMBINE	COMBINE	1200	1.5 – 25.4 (3.64)	26	13	0.98	0.99	0.93
			Dry matter digestibility, %					
EF	EF	498	46 – 71 (4.90)	7	10	1.96	2.02	0.84
PENHAY	PENHAY	381	44 – 72 (6.05)	7	9	1.54	1.63	0.94
PENFRESH	PENFRESH	264	38 – 71 (5.21)	8	10	1.36	1.44	0.93
COMBINE	COMBINE	1143	38 – 72 (5.94)	22	13	1.98	2.03	0.89

[a] Number of samples in the calibration set including outliers. Note that there were multiple samples/cattle for each diet.

[b] Number of samples identified as outliers by the ISI software (critical "T" value of 3, and critical H value of 9) and excluded from the calibration.

Validation. The validation statistics when the calibration equation from one sample set was used to predict diet CP on another sample set provided a useful comparison between calibration approaches and some insight into the limitations of the different methods (Table 2). PENHAY and PENFRESH calibrations predicted EF samples poorly with RMSEP of 1.88 and 2.06, respectively. Similarly, the PENFRESH calibration predicted PENHAY samples poorly (RMSEP of 2.13) whereas the EF calibration predictions on PENHAY and PENFRESH samples were better with RMSEP of 1.56 and 1.06, respectively. All of these independent validations had slopes that deviated from 1.00 by more than 0.10. Williams (2001) recommends that when the slope deviates from 1.00 by more than 0.10 the slope requires investigation as to the cause for the deviation. Such large deviations of the slope from 1.00 usually indicate that validation samples were from different sources than the calibration sample, which was the case for these independent validations. Large deviations of the slope from 1.00 reduce the accuracy of predictions and may contribute to increases in bias and RMSEP.

When the EF, PENHAY, and PENFRESH sets were combined, the SEC and R^2 were similar to those of the PENHAY calibration. Validation of the

combined calibration is considered internal because the validation samples were a subset of the calibration samples. Consequently, all validation statistics improved. RMSEP values for each of the sample sets were much improved (1.00 - 1.13), slopes deviated less than 0.10 from 1.00, and biases were near zero.

Calibration SE values and R^2 values for digestibility in the different sets were comparable with or better than other published results (Lyons and Stuth 1992; Leite and Stuth 1995; Showers 1997; Boval et al. 2004), but of the three methods, SE values were higher and R^2 was lower for the EF calibration. We suggest the poorer EF calibration statistics were the result of greater errors in the reference values. Validation statistics, when the calibration from one sample set (excepting the COMBINE set) was used to predict digestibility of an independent sample set, were unsatisfactory despite SECV being only marginally higher than SEC for the individual calibrations. This indicated a lack of robustness in the EF, PENHAY and PENFRESH calibrations and illustrates the limitations of SECV for validation purposes. When the EF, PENHAY, and PENFRESH sets were combined, the improvement of internal validation statistics was clearly evident (Table 2). We suggest that the combined calibration would have substantially greater robustness than any of the individual method set calibrations.

Table 2. Validation statistics where a calibration equation developed on a designated method set (EF, PENHAY, PENFRESH, COMBINE) was used to predict diet crude protein (CP) and dry matter digestibility (DMD) of diets from a different method set.

Validation set	N[1]	Constituent Range	Calibration Equation	RMSEP	Bias	Slope	r^2
			Dietary crude protein %				
EF	551	3.0 – 19.7	PENHAY	1.88	-0.75	0.76	0.83
			PENFRESH	2.06	1.31	0.89	0.78
			COMBINE	1.06	0	0.96	0.90
PENHAY	393	1.9 – 25.4	EF	1.56	0.19	1.25	0.87
			PENFRESH	2.13	1.00	1.13	0.77
			COMBINE	1.13	-0.13	0.95	0.92
PENFRESH	256	1.5 – 13.9	EF	1.06	-0.56	1.11	0.83
			PENHAY	1.50	0	0.71	0.70
			COMBINE	1.00	-0.06	0.91	0.82
			Dry matter digestibility %				
EF	498	46 - 71	PENHAY	3.69	-1.94	0.74	0.68
			PENFRESH	4.53	2.64	0.69	0.55
			COMBINE	2.43	-0.08	0.95	0.76
PENHAY	381	44 - 72	EF	3.21	-0.60	1.18	0.75
			PENFRESH	3.63	1.63	0.92	0.72
			COMBINE	1.75	0.17	0.95	0.92
PENFRESH	264	38 - 71	EF	4.98	-2.51	1.01	0.44
			PENHAY	4.39	-2.59	1.12	0.63
			COMBINE	2.32	-0.17	1.07	0.84

[1]Number of samples in the validation set.

Strengths and Limitations of Methods for Creating Diet-fecal Pairs

EF method. The main advantage of the EF method was that spectra of the resident cattle would be authentic examples of those from the open population for which calibrations were developed. Furthermore, the chemical composition of the material consumed was not altered in any way by cutting, sun-curing, and storage as occurs during haymaking. These diets, therefore, contrast with the diets of cattle fed in pens where the opportunity for selection is virtually removed and where the diets may differ in many respects from those of grazing cattle.

An additional advantage of the EF method was that sampling strategies could be arranged easily to provide a wide range in constituent values from very low quality to very high quality forage diets. This was achieved by sampling a range of pasture types at different stages of growth from young, lush regrowth through to dry, mature pasture by means of serial sampling throughout the year for a number of years. It was also possible to sample a substantial range of different pasture species and mixtures growing on a range of soil types.

There were also a number of operational disadvantages associated with the EF procedure. First, serious difficulties occurred in obtaining diet-fecal pairs from the full diversity of diets likely to be encountered from cattle grazing an area as extensive and diverse as the northern half of the continent of Australia. The limited availability of EF cattle together with other constraints relating to the care and welfare of such animals, and to labor, transport, and cost factors, meant that the sampling program was restricted to locations within a relatively small area of northeast Queensland and to one site (Brian Pastures) in southeast Queensland. Therefore, to obtain a sufficient diversity of diet types with respect to pasture species and the range of environmental conditions affecting the composition of roughage diets across northern Australia, there was a need to supplement the EF method with pen feeding trials where the diversity of diet types could be expanded by either importing forages from widely dispersed locations or by conducting simple pen experiments at different locations.

Clearly the advantages of the EF method may be negated if mismatch errors are serious (i.e., if diet reference values determined from extrusa samples do not reflect accurately those of the actual integrated diets from which the feces are derived). This is undoubtedly the greatest weakness of the EF method. The previously cited reports of the unreliability of the EF method (Coates et al. 1987; Carulla et al. 1991; Jones and Lascano 1992; Clements et al. 1996; Coates 1999) all dealt with cattle grazing tropical grass-legume pastures where grass-legume proportions in samples of extrusa were often substantially different from those in the diet of the resident cattle. In these experiments, the cattle were confined to uniform swards in small paddocks. Therefore, it is logical to expect that the risk of inaccurate reference values associated with the EF method would increase as paddock size and pasture heterogeneity increased. We believe there are many situations in rangelands where it would be unwise to attempt to determine the diet quality of resident cattle by sampling with EF cattle.

All pastures sampled with EF cattle in the present studies were, in fact, in small paddocks where there was usually a limited

number of pasture species from which the cattle could select. Many of the pastures were grass/legume mixtures, and therefore the relative proportions of grass and legume (in either extrusa or diet) usually had a marked effect on CP concentration. However, as stated earlier, we were able to correct for differences between extrusa and the integrated diet in the proportions of grass (C_4) and legume (C_3) using measured carbon ratios as described by Coates (1999). Without these corrections, many of which were substantial, the calibration statistics for the EF calibration of diet CP would have been much poorer than those presented in Table 1. Despite these corrections, the proportion of calibration outliers, as identified by the ISI software during calibration, was much higher for the EF sample set (4.4%) than for the PENHAY (0.5%) and PENFRESH (1.2%) sample sets. The high incidence of outliers probably was due primarily to substantial errors in the reference values (mismatch errors) of the samples so identified.

Another serious problem associated with the EF method relates to the estimation of digestibility values which necessarily have to be determined by artificial rumen analysis of extrusa. Artificial rumen dry matter disappearance (IVDMD) determinations on extrusa differ from those on the forages from which the samples of extrusa are derived (Coates 1998). The differences occur with both rumen liquor and pepsin-cellulase artificial rumen digestion techniques, and studies conducted at the CSIRO Davies Laboratory in Townsville (Coates, unpublished data) indicate the cause to be associated with the mixing of saliva with the forage and that IVDMD of extrusa is higher than IVDMD of the forage. The effect is quite marked for C_4 grasses but small to negligible for C_3 species tested to date. The magnitude of the difference is linearly and inversely related to digestibility. The incorporation of standards of known live animal digestibility in each artificial rumen run will not overcome the problem; rather it would be necessary to include extrusa samples of the standards of known live animal digestibility. That too would be only partly effective if the unknown samples of extrusa being analyzed were mixed C_3/C_4 diets. Nevertheless, we did correct IVDMD determinations on extrusa using an in-house regression relating IVDMD of extrusa to IVDMD of feed. The regression was developed on a data set which included predominantly C_4 grasses but also some C_3 grasses and legumes. This correction would have been helpful in reducing but not eliminating errors in the reference values caused by the influence of saliva. Therefore, due to potential mismatches between the composition of extrusa and the diet of resident animals and to the problems of measuring digestibility on extrusa, digestibility reference values are likely to be subject to larger than normal errors when the EF method is used to generate diet-fecal pairs from tropical pastures. This would explain why calibration SE values for digestibility in respect of the EF and COMBINE sample sets were larger and R^2 lower than those for the PENHAY and PENFRESH samples sets (Table 1).

PENHAY method. The main advantage of feeding forage hays to cattle in pens is that it is possible to minimize errors in the reference values of the constituents under study. Obviously the choice of hay is important, particularly with respect to the uniformity of composition. Clearly the chemical and botanical composition of the forage offered needs to remain constant over the feeding period to avoid mismatch errors. Rigorous experimental procedures are also extremely important with respect to forage preparation and feeding (e.g., milling the

hay and mixing different hays where appropriate), sub-sampling of forage to provide a bulked sample representative of the forage consumed for later analysis, the health and welfare of the animals, as well as fecal sampling and processing procedures.

Disadvantages include:

- The work required to generate just one diet-fecal pair (with animal replication) is labor intensive and expensive.

- Hays that can be purchased commercially are usually limited to relatively few species, most of which are introduced. Native forbs and browse would occur only as minor contaminants.

- Hays do not represent "natural" diets that free grazing animals would select. In particular, the composition of the feed is altered during the hay making process; the diets are often dominated by a single pasture species; and for tropical grasses, leaf-stem ratios are likely to be lower than in natural diets at similar stages of plant maturity.

- It is very difficult to either purchase or make hays of high protein concentration and high digestibility, especially if the hay is a C_4 grass species. For example, of 28 C_4 grass hays (CP range of 2.25 – 9.50%) that we fed to penned cattle, half the hays had CP < 5% and only 4 had CP > 8%. Those with CP > 8% would probably have been fertilized heavily with nitrogenous fertilizer and, as such, would be dissimilar to diets that cattle would usually encounter on rangelands. Commercially, it is mechanically difficult and uneconomical to cut C_4 grass hay when it is very leafy and lush due to low yield, and the typical product purchased usually has a leaf to stem ratio and quality much lower than diets selected by cattle grazing the grass at a similar stage of growth. Low quality could be overcome partially by custom making batches of hay from specially sown swards that could be cut when very young, but such an option is expensive and would often require hay-making operations to be performed during the wet season when the weather is unsuitable. The reader should note that the high CP and digestibility levels in the PENHAY sample set (Table 1) were achieved by feeding legumes and C_3 grass hays.

PENFRESH method. The main advantage of the PENFRESH procedure over the PENHAY procedure is that the composition of the forage fed directly from the paddock immediately after cutting is not changed by the drying, curing, and storage processes of hay making. As such, the diets (in particular the green diets) were expected to be more akin to grazed diets. The equipment required to harvest standing forage is minimal, consisting simply of a small, tractor-mounted, flail-type forage harvester and trailer. Whereas it may be preferable to feed cattle in separate pens, cattle can be group fed if such facilities are not available. This makes the system very flexible in that the equipment can be moved easily to different properties/locations, and cattle can be fed and watered near to the pasture being harvested in existing or portable yards. This system, therefore, provides the potential for the feeding of a great diversity of diets, especially in relation to geographic diversity and different pasture communities, both native and introduced.

Disadvantages include:

- It is inevitable there will be variation in feed quality between meals despite harvesting from areas of pasture selected for uniformity with respect to botanical composition and stage of growth. This variation may make it difficult to assign

valid reference values to match with fecal spectra for calibration. In our work, we sub-sampled feed offered at every meal during the feeding period and then these samples were analyzed separately so that variation could be monitored. A minimum feeding period of five days is needed for fecal spectra to equilibrate with the pen-fed diet, but we consider 6-10 days feeding to be preferable. Feed quality, especially in C_4 grasses, may change appreciably over the 6-10 day period if the pasture is actively growing, maturing, or under moisture stress.

- A special case of change in feed quality can occur if rain falls during the feeding period. The effect can be most disruptive if the pasture is dry or in a drying cycle and the rain stimulates new growth. There were occasions in our work where trials had to be aborted because of rain.

- The main disadvantage that we encountered was the inability to obtain high quality feed using a tractor drawn forage harvester, even when harvesting relatively immature and actively growing pasture early in the growing season. This was associated with the structure of tropical pastures where diets obtained with a forage harvester contain a disproportionately low leaf to stem ratio compared with diets selected by grazing cattle. The tendency is for forage harvested diets to be low in protein and digestibility. From 50 forage harvested diets at the Brunchilly, Katherine, and Swans Lagoon sites, most of which were harvested when the feed was still green, 76% of the diets had CP < 5%. The highest CP concentrations were 6.8, 8.3 and 6.8% at Brunchilly, Katherine, and Swans Lagoon, respectively. At Lansdown where pastures were prepared by mowing early in the growing season and where N fertilizer was applied, only 7 of 13 forage harvested grass diets had CP > 5%. The highest CP concentration was 10% for a N fertilized and leafy Rhodes grass (*Chloris gayana* cv. Callide) diet that was harvested when regrowth was mainly leaf.

A number of diet-fecal pairs, with animal replication, were generated from PENFRESH diets where all or part of the diet was hand harvested. Hand harvested Para grass (*Brachiaria mutica*) was fed on one occasion while two other trials were conducted where hand harvested *Leucaena leucocephala* leaves and shoots were fed in different proportions (0, 25, and 50%) with a grass hay. Hand harvesting gives excellent control over quality and uniformity, but the labor cost is prohibitive in most situations when feeding large ruminants.

RECOMMENDATIONS

Based on our knowledge of the methods for creating diet-fecal pairs and the results of our work, we believe that each method has its advantages, but each approach also suffers from serious disadvantages. Some of the technical disadvantages associated with the PENHAY and PENFRESH procedures could be overcome if generous resources, particularly labor, were available, but high cost is a disadvantage in its own right. We conclude that none of the three approaches in isolation is capable of generating the required diversity and accuracy of diet-fecal pairs for the development of robust calibration equations to serve an area as extensive and diverse as northern Australia. We consider all three procedures are justified, and perhaps necessary, to build a suitable calibration set.

The PENHAY protocol provides good control over diet quality, and accurate reference values can be matched with fecal spectra. A wide range of constituent values

can be generated if diverse pasture species processed at different stages of growth are used alone or in mixtures (C_3 and C_4 grasses and C_3 pasture legumes). However, with this procedure, there is a problem with obtaining high quality C_4 grass diets, and such diets cannot easily be included in the calibration set if the PENHAY procedure is the only one used. Similarly, most of the native pasture species growing in the rangelands of northern Australia are not readily available as hays. It would be very expensive to prepare suitable batches of hay to provide an adequate representation of the different pasture types across northern Australia. We do not have critical information to indicate whether feed of a certain quality of a particular species can substitute for another species in a calibration set, but we consider that a very diverse calibration set with regard to forage species is necessary for robust calibration.

The PENFRESH procedure enables fresh, green feeds to be represented in the calibration set while at the same time providing for accuracy in reference values in most instances. Variation in diet quality through the feeding period can be monitored easily, and the risk of assigning inaccurate reference values to fecal spectra can be assessed and a decision can be reached as to the acceptance or rejection of a diet-fecal pair. Once again we have no critical information to indicate whether F.NIRS calibration equations developed solely from feeding cattle forage hays are likely to be less than satisfactory for predicting diet quality when cattle graze fresh feed. However, it was apparent that RMSEP values were disappointingly high when the PENHAY calibration was used to predict diet CP and digestibility of the PENFRESH and EF sample sets (Table 2). In general, mechanical harvesting of forage is needed to supply the required quantity of forage while keeping costs within reasonable limits. This has consequences on the quality of forage harvested from tropical grass pastures such that the reference values for both diet CP and digestibility of most PENFRESH diet-fecal pairs in our work fell at the lower end of the range. Therefore, while the PENFRESH procedure based on mechanically harvested forage is useful, it needs to be supplemented with other methods. As with the PENHAY method, the technical problems of the PENFRESH procedure could be overcome if unlimited labor allowed diets to be hand harvested where necessary. Hand harvesting diets would, of course, be much more practicable for small ruminants such as sheep and goats.

The overriding disadvantage of the EF procedure is the risk of inaccurate reference values due to 1) mismatches in diet-fecal pairs and 2) for diets containing C_4 grass, problems in obtaining valid measures of digestibility by artificial rumen techniques on samples of extrusa. Nevertheless, we have no doubt that the EF method made a valuable contribution to our calibration set and to the quality of the calibration equations we developed. In particular, the EF method provided most of the high quality diets composed of tropical pasture species in our calibration set. Without the contribution of the EF method, high quality tropical pasture diets would have been very poorly represented in the calibration set, and this would almost certainly have resulted in much poorer robustness. Nevertheless, because accuracy of reference values is of such importance in developing any NIRS calibration equation, we suggest that the EF protocol should be used with caution and only in those situations where the risk of extrusa composition being different from the integrated diet is minimal or where differences can be detected and corrected.

Current Status of Calibration Equations

Although the COMBINE calibration set currently contains 1200 fecal spectra with diet CP reference values and 1143 fecal spectra with DMD reference values, the fecal spectra were derived from only 315 and 285 different diets for diet CP and DMD, respectively. Moreover, although the progressive accumulation of diet-fecal pairs required an enormous amount of effort and expense, we consider the calibration set to be small relative to the target area for which the technology is being developed and inadequate for some applications. Nevertheless, we feel confident that current calibration equations provide predictions of sufficient accuracy for many of the pasture systems across northern Australia to be useful for a range of purposes such as:

(a) F.NIRS predictions of diet quality and other parameters as decision support for graziers and/or consultants in the nutritional management of grazing cattle and the management of pasture.

(b) F.NIRS predictions as a powerful educational tool to help producers, consultants, agribusiness, students, extension personnel and scientists obtain a better understanding of the pasture and vegetation resources of Australian rangelands, particularly with respect to nutritional dynamics and limitations.

(c) F.NIRS as a research tool for scientists in the conduct of grazing experiments.

Regardless of the method used to generate diet-fecal pairs, reference errors for diet quality constituents will be higher for F.NIRS than for the equivalent constituents in NIRS forage analysis due to some degree of mismatch between the sample analyzed to provide diet reference values and the actual integrated diet of the cattle producing the feces. Unfortunately it is not possible to determine to what extent calibration and/or validation statistics are adversely affected by mismatch errors, but it is likely that such errors would be substantial for some of the diets. We have argued that mismatch errors are likely to be larger and more frequent for the EF method than for the PENHAY and PENFRESH procedures and the effect was reflected in the calibration statistics, either as the frequency of outliers or higher SE values. It is also likely that for samples identified as outliers and those not identified as outliers but with high residuals (reference minus predicted) many probably suffered from substantial mismatch errors. We base this contention on a consideration of the relevant diets and an assessment of potential mismatch errors in relation to the type of diet being fed or pasture being grazed. Because there are grounds for believing that it is possible for NIRS predictions to be more accurate than laboratory reference values when reference errors are random (Coates 2002; DiFoggio 1995), the calibration statistics presented in Table 1 may underestimate potential correlations between actual and predicted values.

The same argument holds true of the validation statistics presented in Table 2, especially because outliers identified during calibration were not eliminated from the sample sets. Although random reference errors will have the effect of increasing residuals in some instances but also reducing residuals in other instances, the overall effect of higher reference errors due to the mismatch phenomenon will be poorer validation statistics. Therefore, we contend that the RMSEP and r^2 validation statistics shown in Table 2 are influenced negatively as a result of reference value errors, particularly mismatch errors.

However, we also are aware of deficiencies within the calibration set, deficiencies due to some important types of diet being unrepresented or inadequately represented in the current calibration set and the probable or possible consequences of these deficiencies, not only on the accuracy of the predictions but also on the nutritional interpretation of predictions.

Diets not represented or inadequately represented relate primarily to those where the use of the EF method would be inappropriate due to the high risk of potentially large mismatch errors and/or where it is difficult and expensive to obtain the desired diet types in sufficient quantity to feed to cattle in feeding trials. Three such diet types are:

(i) diets that contain a high proportion of native forbs such as is common in the vast Mitchell grass (*Astrebla* spp.) areas of northern Australia,

(ii) diets that contain moderate or high levels of native browse, especially where the browse is high in condensed tannins such as in Mulga (*Acacia aneura*) and other *Acacia* spp.,

(iii) diets in the infertile arid and semiarid areas where Spinifex (*Triodia* spp.) is a common component.

High Forb Diets Typical of Mitchell Grass Country. Numerous forb species grow prolifically on the fertile Mitchell grass plains of western Queensland, the Northern Territory and parts of Western Australia. Many of the forbs are palatable and have high nutritive value (protein and ME content), higher than the perennial grasses and mature annual grasses, and they are a vitally important forage resource for grazing livestock in the Mitchell grass areas. We had hoped to obtain diets with a high forb component in the PENFRESH feeding experiments at Brunchilly, but the forbs typically are located in the lower stratum of the pasture profile, growing between grass tussocks so that harvested forage contained but a small amount of forbs. Harvesting closer to the ground in an attempt to obtain a greater proportion of forbs merely increases the proportion of coarse grass stems. Occasions do occur in some locations and at certain times in some years where the growth of forbs would make it possible to mechanically harvest forage with a high percentage of forbs, but such occasions rarely coincide with the availability of suitable research personnel, equipment, and funding. Hand harvesting of forbs is technically feasible but not normally a practical option in terms of cost.

Browse Diets. The foliage and shoots of native browse shrubs and trees contribute widely to cattle diets in the savannas and shrublands of northern Australia. Many native browse species are characterized by the presence of anti-nutritive compounds such as condensed tannins (Everist 1986), and cattle generally commence browsing when the availability or nutritive value of grasses and forbs declines to certain threshold levels in the dry season or during droughts. Two problems exist regarding the generation of diet-fecal pairs for browse-containing diets. The first relates to difficulties in obtaining sufficient fresh material to feed to cattle in pens where hand harvesting appears to be the only practical option. Harvesting, drying and storing foliage to build up feed stocks over time does not appear to be a viable option because of the chemical changes that occur during drying and the effect of such chemical changes on the anti-nutritive properties of the feed (Mahyuddin et al. 1988; Palmer and Schlink 1992; Palmer et

al. 2000). The second problem relates to the probable need to carry out the trials using cattle already adapted to the browse feeds, and this would necessitate undertaking the feeding experiments where the feed grows (generally remote) and at times when cattle would be browsing by choice.

A third and associated problem relating to diets with browse and where the browse contains high concentrations of condensed tannins is the interpretation of the F.NIRS prediction of diet CP. Because condensed tannins can adversely affect protein availability, knowledge of total CP is not likely to be very helpful without some estimate of availability as well. We believe this area of research warrants serious attention in Australia, and we view with great interest the work being conducted by Landau and colleagues (Landau et al. 2004).

Spinifex Diets. We single out Spinifex grasses because of the special characteristics such as high silica content that could influence F.NIRS predictions and because there are extensive areas across arid and semiarid Australia where Spinifex grasses are an important part of the forage resource. As yet, Spinifex is not represented in our calibration set, but Spinifex diets warrant adequate representation. The current non-representation of Spinifex diets can be attributed to remoteness and harvesting difficulties.

Monitoring Performance

Creating validation sets of diet-fecal pairs and monitoring the performance of F.NIRS calibration equations for predicting diet quality is, like the initial development of equations, a tedious and expensive process. The short term consequence in Australia is that the process has stalled due to the lack of ongoing research funding. We are hopeful of opportunities that may arise from pen experiments designed and conducted for purposes other than NIRS research where, for very little or no extra effort, samples of diet and feces could be made available to assist in the monitoring and expansion of F.NIRS technology.

ACKNOWLEDGEMENTS

We wish to acknowledge the pioneering work of Jerry Stuth and his colleagues and his encouragement to develop F.NIRS technology in Australia. We also thank Meat and Livestock Australia for funding assistance and we wish to acknowledge the assistance of the many co-operators who assisted us in our research.

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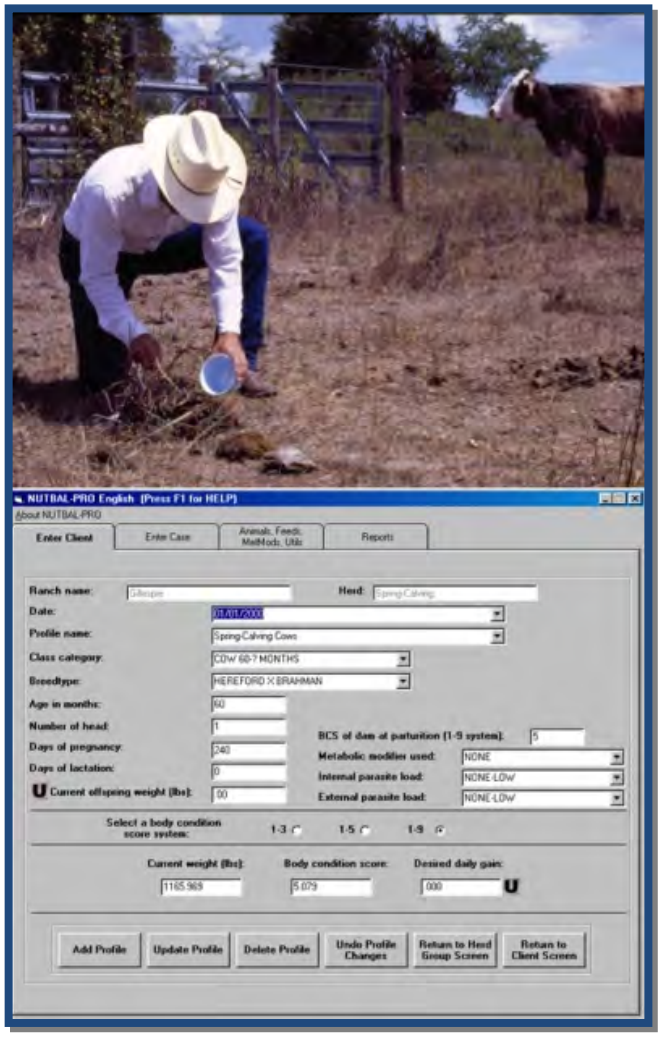
Chapter 4. A Locally Adapted Method for Improving Fecal NIRS and NutBal-PRO Predictions of Cattle Performance

Robert K. Lyons

Objectives: To show how F.NIRS diet quality predictions can be used with nutritional models to predict cattle performance, and how adjustments can be made to improve future predictions when those model predictions do not meet actual performance.



Key Points

- Independent validation studies and agreement between seasonal F.NIRS predictions of diet quality show that F.NIRS predicts diet quality of free-grazing cattle fairly accurately.
- Using the F.NIRS predictions of CP and DOM in the NutBal-Pro nutritional model with no adjustments often resulted in poor weight gain and BCS predictions.
- Under-predicting was corrected by using metabolizable protein instead of crude protein and by eliminating constraints on intake caused by high temperatures and low CP.
- Over-predicting was corrected by reducing intake until model predictions matched actual performance using a moving average so that the model coupled with the F.NIRS predictions accurately predicted future performance.
- This system can be used to tell if animal performance is limited by forage quality or forage quantity.

INTRODUCTION

A major reason for developing F.NIRS equations to estimate forage quality is to use these estimates to predict animal performance and improve grazing animal nutritional management. A computer model is a convenient way to predict performance of various kinds and classes of livestock using these forage quality estimates.

Rangelands with their forage species diversity, variability in forage biomass production, and extensive management scale present a particular challenge in obtaining adequate information for use in nutritional models. Specifically, on rangelands, estimating the portion of the standing crop grazing animals will consume and, therefore, estimating forage intake and diet quality is difficult. For example, studies have reported 80% of the diets of grazing animals originating from 1 to 6% of the standing crop (Arnold and Dudzinski 1978; Cruz and Ganskopp 1998). Kirby and Stuth (1982) reported that 85% of cattle diets were from two grasses during three seasons. In addition, O'Reagain and Grau (1995) reported that tillers from least-preferred grass species were not grazed until 80 to 100% of the tillers from the preferred and intermediate species were defoliated.

A reasonable estimate of forage intake is needed to provide acceptable predictions of animal performance from nutritional models, and nutrient intake is a function of diet quality and quantity. Researchers have reported that, coupled with estimates of diet quality from hand-plucked samples, animal performance can be a useful tool for estimating forage intake on a group or pasture-basis (Moore 1996) and with grazing, lactating dairy cattle (Macoon et al. 2003). When diet quality and performance are known, intake can be estimated; therefore, we hypothesized that by adjusting the intake estimates from nutritional models to match performance we could enhance the value of using F.NIRS estimates of diet quality combined with nutrient models to make management decisions.

This chapter presents evidence of potential fecal NIRS accuracy, examples of nutritional model-use with F.NIRS estimates, and examples of use of animal performance to calibrate forage intake for individual ranches to 1) improve model performance estimates and 2) differentiate forage quality and quantity problems.

Evidence of Fecal NIRS Accuracy

In 1995, Lyons et al. reported results of validation trials for NIRS fecal equations developed for cattle (Lyons and Stuth 1992). In these validation trials, forage samples were collected at the beginning of each trial with esophageal-fistulated steers and fecal samples were collected from cows introduced to sampled areas. Cows grazed native grasses in six of these trials and ryegrass in a seventh trial. In this study, the relationship between forage crude protein (CP) and digestible organic matter (DOM) and F.NIRS estimates of forage CP and DOM were compared at 12-hour intervals from 0 to 72 hours after cows began grazing trial pastures. While all parameters (simple coefficient of determination [r^2], intercept, and slope) indicated that F.NIRS predictions of forage CP and DOM were not statistically different from actual forage values at 60 hours after entering a trial pasture, the best match occurred at 72 hours (Table 1). A by-trial comparison of mean forage CP and DOM with 72-hour F.NIRS determinations (Figures 1 and 2) illustrates the similarity between NIRS predictions and sampled-

forages. Other studies (Andrae et al. 2000; Lalman et al. 2001; Mattox 2001; Horsley 2002) have indicated that F.NIRS tended to overestimate diet quality and thus animal performance.

Table 1. Independent validation statistics for F.NIRS calibrations developed by Lyons and Stuth (1992) for forage sample crude protein and digestible organic matter. Adapted from Lyons et al. (1995).

Hour[a]	r^2	Intercept	p-value[b]	Slope	p-value[c]
		Crude Protein			
0	0.18	8.2	0.0200	0.29	<0.005
24	0.53	7.0	0.0190	0.36	<0.005
36	0.88	4.7	0.0159	0.60	<0.005
48	0.97	2.2	0.0618	0.82	<0.025
60	0.96	2.5	0.2481	0.89	<0.100
72	0.98	-0.1	0.9283	0.98	<0.250
		Digestible Organic Matter			
0	0.02	55.2	0.0118	0.07	<0.005
24	0.29	43.2	0.0172	0.29	<0.010
36	0.75	26.4	0.0320	0.58	<0.025
48	0.87	14.9	0.1171	0.77	<0.100
60	0.87	5.3	0.6090	0.92	<0.250
72	0.87	2.4	0.8157	0.97	<0.250

[a]Hour fecal samples were collected from the time grazing cows were introduced to trial pastures.
[b]Probability that the intercept is not different from 0.
[c]Probability that the slope is not different from 1.

Anecdotal evidence from forage quality determinations performed at the Grazing Animal Nutrition Lab at Texas A&M University over a 10-year period, implies that F.NIRS tracks seasonal trends as well as variability in forage quality for the Edwards Plateau region of Texas (Figure 3). Peak estimated CP and DOM occurred in April with another minor peak in September for CP. These peaks are consistent with the bi-modal rainfall pattern for this region. Values seen in July are consistent with summer dormancy. In addition, maximum and minimum F.NIRS estimates of forage CP (Figure 4) are consistent with the kinds of forages grown in the region.

Integrating NIRS Fecal Analysis with a Nutritional Model

A major reason for developing NIRS equations to estimate diet quality from fecal analysis is to provide ranchers the information necessary for nutritional management of free-grazing livestock. Interpreting results of NIRS fecal analysis for specific physiological stages, environmental conditions, and forage availability requires multiple calculations which are best accomplished with a computer model. Fox (1995) suggested that nutritional models developed to predict beef cattle performance require 1) adequate information to drive these models and 2) user understanding of underlying concepts to adjust models to unique farm or ranch animal, environmental, feed, and management factors.

To test the accuracy of NIRS fecal analysis coupled with a nutritional computer model, a study was conducted by Lyons and Machen (2007) in 6 cow-calf herds on five Texas ranches. Forage diet quality in terms of crude protein (CP) and digestible organic matter (DOM) was estimated from NIRS-analysis of composite fecal samples taken each month of the study period for each ranch. Samples were analyzed by the Grazing Animal Nutrition Lab at Texas A&M University. In this study, herd and environmental information and diet quality estimates were entered in the NutBal-PRO nutritional model (Ranching Systems Group 2002) to predict cow performance over the next 30 days. As a measure of performance for model validation, cows were body condition scored on a 1 to 9-basis (Herd and Sprott 1986) each month at fecal sampling.

Computer nutritional models can either: 1) estimate performance (i.e., weight of BCS change) accurately, 2) over-estimate performance, or 3) under-estimate performance. All three of these situations

were encountered during the Lyons and Machen (2007) study. Sources of errors when integrating F.NIRS determinations of diet quality with NutBal PRO can be 1) knowledge gaps related to basic model assumptions and equations including incorrect estimates of forage intake, or 2) incorrect estimates of diet quality.

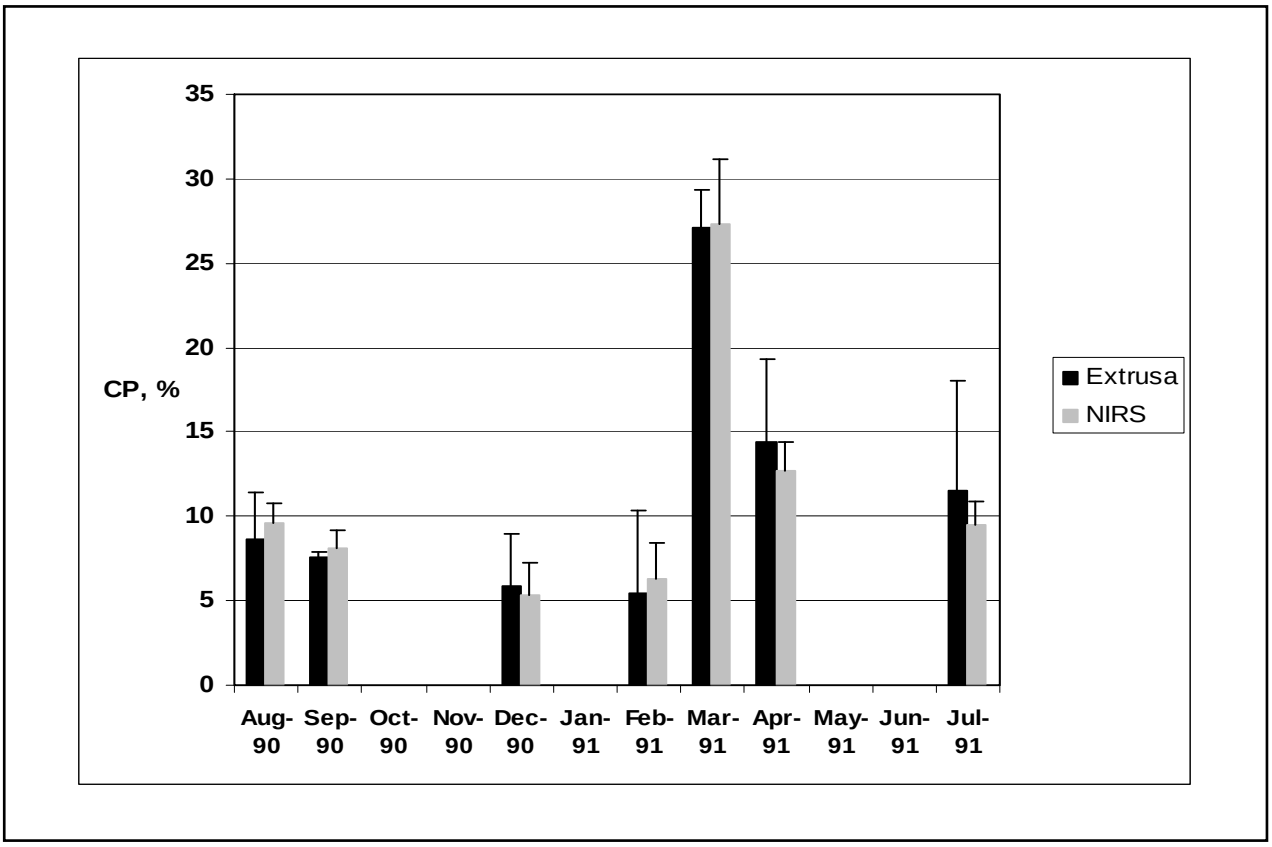


Figure 1. Comparison of diet (extrusa) crude protein (CP) values obtained with esophageal-fistulated steers and forage CP predictions from NIRS fecal analysis 72 hours after introduction of cows to trial pastures. Mean values are shown with 95% confidence intervals. Adapted from Lyons et al. 1995.

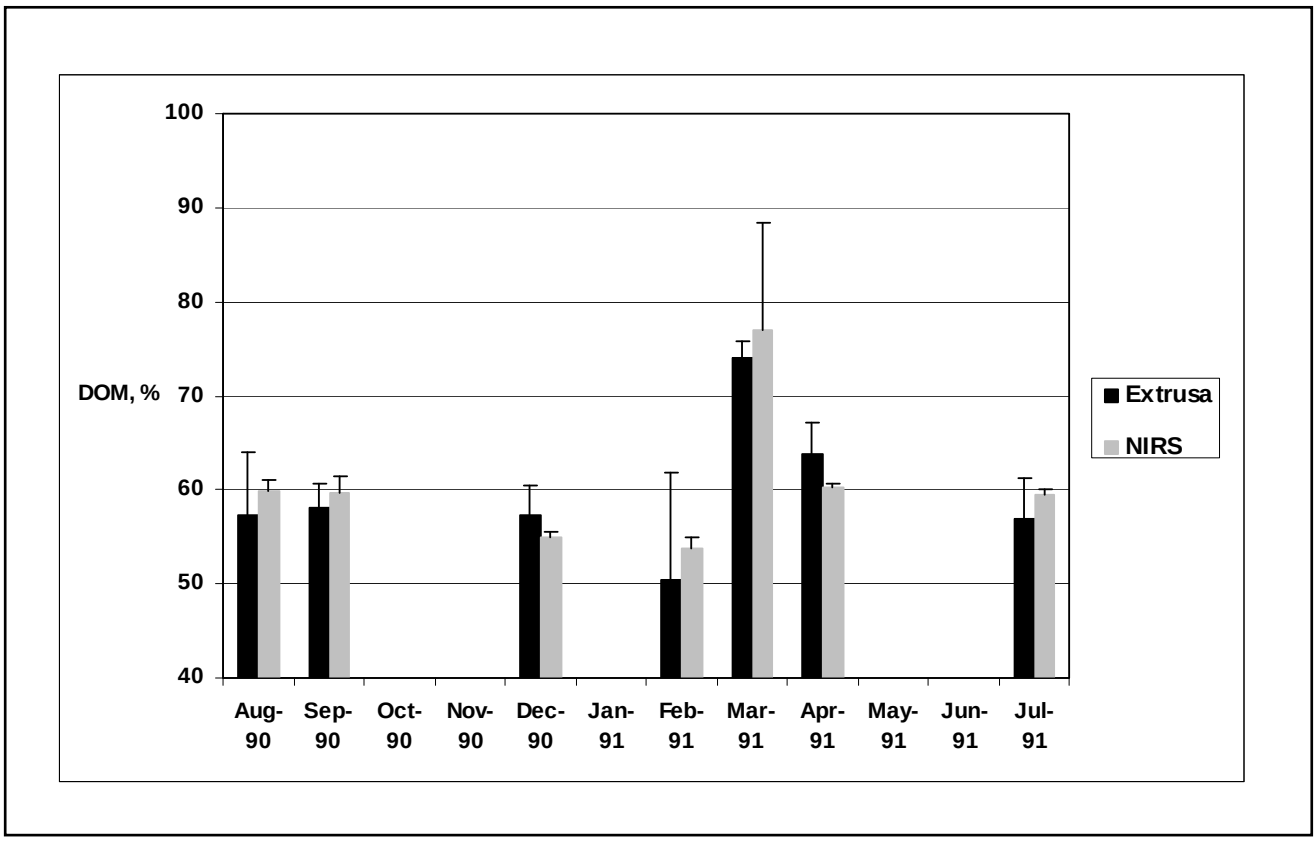


Figure 2. Comparison of diet (extrusa) digestible organic matter (DOM) values obtained with esophageal-fistulated steers and forage DOM predictions from NIRS fecal analysis 72 hours after introduction of cows to trial pastures. Mean values are shown with 95% confidence intervals. Adapted from Lyons et al. 1995.

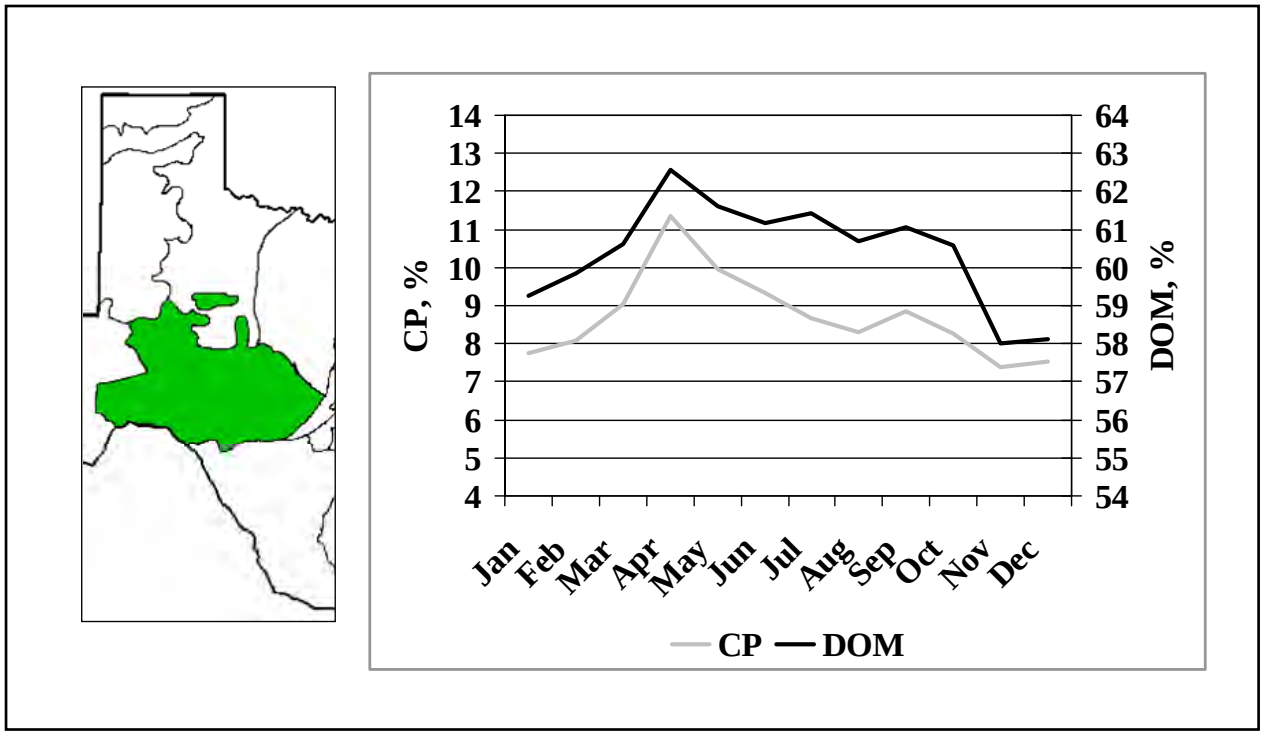


Figure 3. Seasonal trends in crude protein (CP) and digestible organic matter (DOM) predictions from NIRS fecal analysis for the Edwards Plateau region of Texas over a 10-year period (Grazing Animal Nutrition Lab, Texas A&M University).

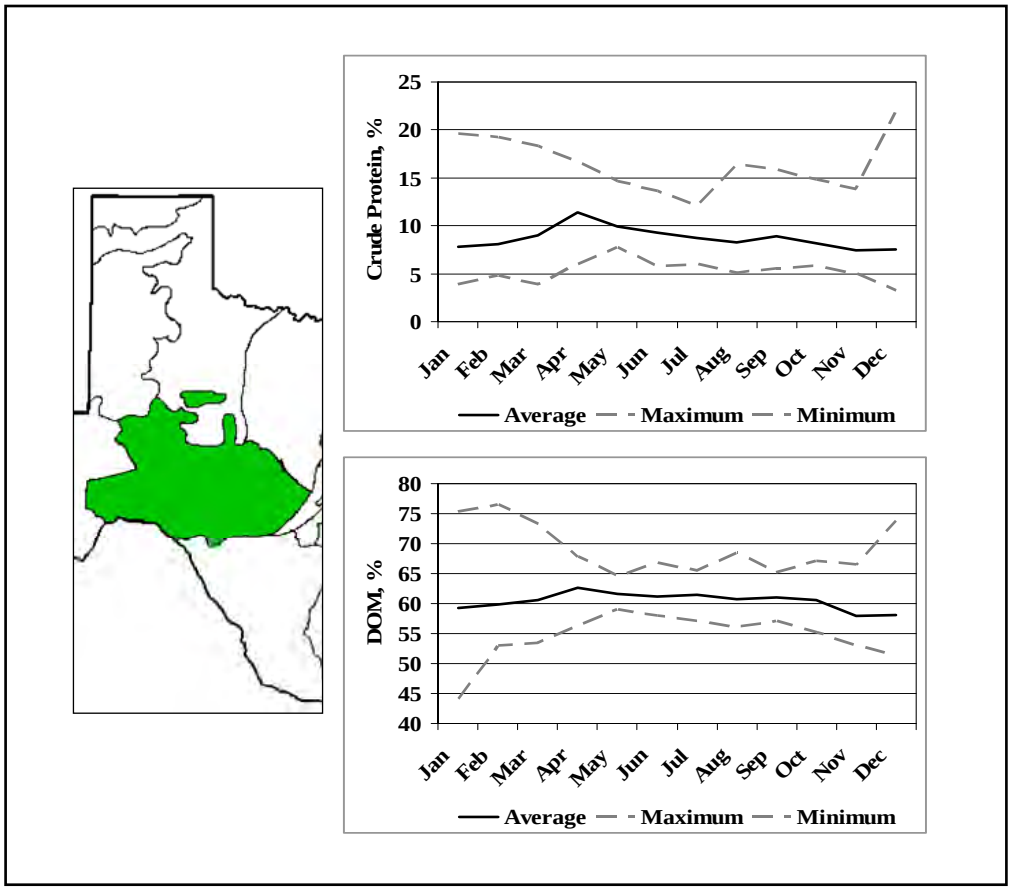


Figure 4. Maximum, minimum, and average forage crude protein (CP) and digestible organic matter (DOM) estimates from NIRS fecal analysis for the Edwards Plateau region of Texas over a 10-year period (Grazing Animal Nutrition Lab, Texas A&M University). Maximum and minimum values in winter months are indicative of cool-season annual and dormant warm-season perennial grasses.

Under-estimating Performance

Under-estimates of performance (Lyons and Machen 2007) were related to three sources. First, a model equation reduced forage intake when diet CP was below 6%. Because the model uses diet digestibility and fecal output to estimate basic forage intake and because digestibility declines as CP declines, the below 6% CP equation implemented a reduction in forage intake in addition to that associated with decreasing digestibility. This error source was corrected by removing the CP driven forage intake reduction equation from the model. A second error source was a reduction of intake caused by high environmental temperatures. This source was eliminated by limiting maximum temperatures to 29^{o} C (85^{o} F). For cattle in this study, temperatures above this level tended to overestimate condition score loss. A third source was the use of crude protein versus metabolizable protein-based performance estimates. For low-protein forages, crude protein-based estimates under-estimated performance compared to metabolizable protein-based estimates (Table 2). For low-protein forages, provided there is adequate energy in the rumen, the metabolizable protein basis (National Research Council 1996) yields a higher estimate of post-ruminal protein available for digestion, and thus, a higher predicted performance.

Table 2. Comparison of body condition score predictions with the NutBal PRO computer nutritional model using crude protein versus metabolizable protein approaches. Comparisons are for instances where the crude protein approach underestimated cow body condition scores.

Treatment	BCS
Observed	5.2[a]
MP-Predicted	5.1[a]
CP-Predicted	4.4[b]

[a,b]Mean values (n=15) followed by different letters are significantly different ($P < 0.05$).

Over-estimating Performance

In grazing situations, especially rangelands, a major source of error for computer-model-predicted performance is the amount of available forage, or more precisely, the amount of forage preferred by the grazing animal (Arnold and Dudzinski 1978; Kirby and Stuth 1982; O'Reagain and Grau 1995; Cruz and Ganskopp 1998). Lyons and Machen (2007) approached the problem of available or "preferred" forage by using cow body condition score (BCS) both to validate model performance predictions and as a basis for deriving an estimate of apparent forage intake. Model-predicted body condition scores using unadjusted forage intake were compared to observed condition scores. When BCS gain was overestimated and all other model entries appeared correct, the model was rerun reducing potential forage intake by percentages until model and observed BCS matched. Adjusted forage intake required to match observed BCS was recorded as apparent forage intake and used to create a continuous average forage intake value. Continuous average intake was determined by averaging apparent forage intake for all previous months. The current continuous value was used in the model to predict BCS for the next month.

NIRS Fecal Analysis/Nutritional Model Integration Case Study

In the Lyons and Machen (2007) study, BCS estimates based on unadjusted forage intake were greater than either observed BCS or estimates using continuous average intake values ($P < 0.0001$). However, body condition score estimates based on continuous average forage intake did not differ ($P = 0.7757$) from observed BCS, which points to the potential and value of calibrating nutritional models to individual ranches.

Average unadjusted daily forage intake (Lyons and Machen 2007) among ranches ranged from 26 to 32 pounds. Average un-adjusted intake ranged from 2.4 to 2.8% of body weight on a condition score 5 basis. A reasonable range relative to a traditional animal unit is an average daily intake level of 26 pounds for a 1000-lb cow or 2.6% of body weight. In contrast, average apparent forage intake (Lyons and Machen 2007) ranged from 19 to 24 pounds across ranches or 1.7 to 2.1% of body weight with an average of 2.0%. Maximum average apparent forage intake was 2.7% across ranches, which was close to average expected intake. Maximum and minimum apparent intake was 3.1% and 1.1% of body weight, respectively. In comparison, Holechek et al. (2001) reported average dry-matter intake of about 2% of body weight for grazing cattle, ranging from 1.2 to 2.8%. Pinchak et al. (1990) reported values for cattle of 1.95 to 2.45%.

Other studies (Andrae et al. 2000; Lalman et al. 2001; Mattox 2001; Horsley 2002) have attributed over-estimates of animal performance to a tendency for fecal NIRS to overestimate diet quality. Although these potential over-estimates are certainly a source of error, they do not account for all of the error in the Lyons and Machen (2007)

study. In the Lyons et al. (1995) validation study, the extrusa DOM/fecal NIRS-estimated DOM regression intercept was 2.4 percentage units. Reducing fecal NIRS DOM estimates by 2.4 percentage points would result in a reduction in forage intake of about 6%. However, in the Lyons and Machen (2007) study, the average forage intake adjustment necessary to match observed BCS was about 22% with a range of 0 to 64%.

Nutritional Applications & Implications

Fecal near infrared reflectance spectroscopy analysis provides a convenient method of estimating forage quality for grazing cattle. Combining F.NIRS analysis with 1) a computer model like NutBal-PRO to interpret fecal analysis results and 2) a performance estimate such as body condition scoring to adjust the model to local ranch conditions including animal adaptation to temperatures and amount of preferred forage available provides a nutritional analysis system. This system can be used to distinguish between forage quality and forage quantity as limiting nutritional factors.

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Chapter 5. Fecal NIRS for Predicting Botanical Composition of Herbivore Diets

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Objectives: To describe advantages and disadvantages of using F.NIRS for predicting botanical composition of the diets of free-grazing animals and how to conduct calibration trials to get the best results.

Key Points

- F.NIRS determines relative differences between diets accurately on most samples. However, unless calibrations are developed on the same diets as samples to be predicted, the predictions will provide only approximate values of the percent of a plant in the diet.
- Microhistological analysis of fecal samples is less accurate than F.NIRS and cannot be used to develop calibrations or monitor calibration performance.
- Calibration equations can be enhanced by increasing the diet diversity fed with the target plant.
- Guidelines for conducting a feeding trial to create diet-fecal pairs include
  - Air dried target plants can be used in feeding trials.
  - Two to three levels of a target plant plus zeros are adequate for calibration.
  - Diets should be fed for 4 days before collecting fecal samples.
  - Sex of animal affects calibrations.

INTRODUCTION

By preferring some plants and avoiding others, livestock have a profound impact on the botanical composition of plant communities. The use of selective grazing by different livestock species and manipulation of grazing preferences by various management practices are important techniques for managing unwanted plant species (Launchbaugh et al. 2006). Research to quantify botanical composition of the diet of free-grazing herbivores has been hindered by a lack of adequate methods for determining diet composition. For instance, Harniss et al. (1975) reported that for sheep grazing sagebrush-bunchgrass rangelands, 150 fistula diet determinations would be necessary to estimate the percentage of plant species that comprise 20% of the diet to within 10% of the mean with a 95% probability. Such sampling intensity exceeds the practical capacity of currently available methods for determining botanical composition of diets except fecal near-infrared reflectance spectroscopy (F.NIRS). However, if F.NIRS for determining botanical composition of diets is to be accepted, the precision, accuracy and sources of variation of determinations must be understood.

The objective of this chapter is to show the value and limitations of F.NIRS for determining the percentage of a target plant in an herbivore's diet. We will show the effect of including samples with none of the target species (i.e., zero fecals) and how levels of target plant and diversity of background diets affect calibrations. The effect of other factors such as age and sex of animals or fresh vs. dried forages, which may affect the precision and accuracy of determinations, also will be demonstrated.

MATERIALS and METHODS

Calibrations and validations reported in this chapter are based on diet-fecal pairs obtained from feeding trials designed to develop calibrations for determining the target plant species leafy spurge (*Euphorbia esula* L.), spotted knapweed (*Centaurea biebersteinii* DC.), mountain big sagebrush (*Artemisia tridentata* Nutt. Spp. vaseyana [Rydb] Beetle), and redberry (*Juniperus pinchotii* Sudw.) or ashe (*J. ashei* Buchh.) juniper that were ground through a 2.5 cm screen and mixed with other forages, mostly alfalfa (*Medicago sativa* L.) and various grass hays, to make a mixed diet.

Leafy spurge

In 1992, diets containing 15, 30, 45, 60, 75 and 88% leafy spurge with the remainder of the ration either alfalfa or smooth brome and barley straw mix were fed to 20 sheep and 20 goats. In 1994, 10 each sheep and goats were fed alfalfa hay at 0.5% of body weight with free choice access to leafy spurge hay from two sources (Idaho or North Dakota). Percentage of leafy spurge in the diet was the average percentage of leafy spurge consumed 48 and 72 hours before feces were collected. Data for sheep and goats were combined for the analyses presented (see Walker et al. 1998 for details of both trials).

Spotted knapweed

In 2005, spotted knapweed from several sources was fed to sheep at 0, 10 and 50% of the diet with several combinations of alfalfa or grass hay as a background for a total of 12 different knapweed background combinations, resulting in a total of 36 diet-fecal pairs. In 2006, spotted knapweed was fed at 0, 15 and 30% of the diet with four different backgrounds. In a second trial, spotted knapweed was fed at 15, 19 and

25% of the diet but with varying levels of intake such that total knapweed consumption was the same for the three different percentages, and there was a single alfalfa and grass hay mixture as the background forage. Data from the two trials conducted in 2006 were combined.

Sagebrush

In 1996, diets containing 0, 4, 8, 12, 16 or 24% mountain big sagebrush with base diets of alfalfa/grass hays in the proportions: 0:100, 20:80, 40:60, 60:40, 80:20 or 100:0 for a total of 36 diets were fed to mature ewes. In 1998, diets containing 0, 8, 16 or 24% of either air dried or frozen sagebrush with a 1:1 mix of alfalfa and grass hay background were formulated for a total of seven diets. Each diet was fed to five replicated lambs (see Walker et al. 2002 for complete details).

Juniper

In 1999, 16 10-month old Boer-Spanish cross wether goats were fed for three consecutive 8-day feeding periods in which the background diets varied. The background diets consisted of Coastal bermudagrass hay (*Cynodon dactylon* (L.) Pers.) for period 1, alfalfa hay for period 2, and a 1:1 mix of bermudagrass and alfalfa hays for period 3. Redberry juniper needles were mixed with the background forages to create a final diet with 0, 5, 10, 15, 20, 30, 40 and 50% juniper on a dry weight basis. Two replicate goats were assigned randomly to each diet. In 2002, 16 9-month-old Boer-Spanish cross wether goats were allocated randomly to 16 combinations of two different juniper types and eight basal diets. The juniper types were either pure ashe juniper or a 1:1 mixture of ashe and redberry juniper. The eight basal diets consisted of (1) alfalfa hay, (2) peanut hay, (3) Coastal bermudagrass hay, (4) sudan hay, (5) ryegrass hay, (6) wheat hay, (7) a mixture of native forbs, or (8) a mixture of the hays plus 10% native browse consisting of live oak (*Quercus virginiana* Mill.) and fourwing saltbush (*Atriplex canescens* (Pursh) Nutt.). There were three periods of 8 days in which animals were fed successively the basal diet plus 0, 10 or 40% juniper. In 2004, 11 goats in each of the following breed, gender, and age groups were used in this experiment: female Angora, intact male Angora, castrated male Angora, female meat goats, intact male meat-type goats (Boer x Spanish), castrated male meat goats, Angora female kids, and Angora male kids for a total of 88 goats. Two complete mixed pelleted diets containing 0 or 14% juniper were fed to goats of differing breed, gender, and age. Dried and ground leaves were added to a commercial complete mixed goat diet to create a diet that contained 14% juniper. The diets were pelleted to prevent ingredient sorting when goats were fed the 14% juniper diet. For complete details of the 2004 feeding trial, see Walker et al. (2007).

Fecal samples were dried in a forced air oven at 55°C for 24 hours, ground in a cyclone mill to pass through a 1 mm screen, dried again as above and conditioned for 24 hours in an environment with constant temperature and humidity (21°C and 65%, respectively). Samples were then packed into sample cells with a near-infrared transparent quartz lens. Cells were scanned 32 times using a scanning reflectance monochromator (model 6500, Foss NIRSystems, Inc., Silver Springs, MD). Reflected energy (log 1/R) was measured and averaged over the 32 scans and recorded at 2-nm intervals from 400 to 2500 nm, but only wavelengths greater than 1100 nm were used in calibrations because experience showed that the shorter wavelengths did not increase precision or accuracy of predictions. Theoretically the longer wavelengths are related more closely to the

chemical bonds in the sample than those in the visible spectra, and reducing the number of wavelengths is one way of increasing model stability (Naes et al. 2002). Replicate daily spectra for animals on the same diet were averaged before calibration. Prior to calibration development, spectra were pretreated with an eight segment moving average then taking a second derivative over an eight segment gap. Scatter correction was not used. Calibration equations using derivatized spectra were developed using modified partial least squares (MPLS) equations.

Fecal samples from selected diets from the 1992 leafy spurge trial and the 2002 juniper trial were sent to the Wildlife Habitat and Nutrition Laboratory at Washington State University for microhistological analysis. Because of the cost of this analysis, a subset of samples was selected to represent the range of concentration of the target plant and the background forages. For the leafy spurge and juniper samples, two and four slides per sample, respectively, were prepared, and 25 fields per slide were viewed.

In general, the comparisons of different calibrations were done by comparing simple coefficients of determination (r^2), slope (β), root mean square error of prediction (RMSEP) and standard error of cross validation (SECV). The r^2 is an indicator of the precision of the determinations. Slope is the change in F.NIRS value for a unit change in the laboratory value and is an indicator of accuracy of predictions. When $\beta < 1$, it indicates the calibration has decreased the variation in predicted values relative to laboratory values, and when $\beta > 1$ the opposite is true. RMSEP is an indication of overall error of independent predictions that includes error in both precision and accuracy. SECV is similar to RMSEP except that it is based on calibrations using a portion of the samples to predict the remaining samples and does not represent the error expected for independent predictions.

To evaluate the usefulness of calibrations when applied to samples that were collected from free-grazing animals, independent validations were conducted by developing calibration from one feeding trial and validating these calibrations with the samples from the other feeding trial. This was done reciprocally so that data from each trial served for both calibration and validation. The calibration that had the highest r^2 for the independent validation was designated the "best" calibration, whereas the other calibration was designated "worst." In actual practice where the two calibration data sets would be combined to predict independent samples, the actual results would be expected to be improved because the larger data set would include a wider range of variables and thus be more robust.

RESULTS and DISCUSSION

Previously published F.NIRS calibration statistics for botanical composition of the diet have all shown good calibration statistics with r^2 generally greater than 0.9, slopes close to unity, and SECV less than 5 percentage units. With the exception of calibrations for alfalfa and grass in juniper calibration trials, Table 1 shows similar results for the four target plants and two background forages. Each of the calibration data sets consisted of diet-fecal pairs from two independent feeding trials conducted in separate years with different sources of forages and often different experimental designs relative to background forages and levels of target plants. The calibration statistics for the combined data sets were similar to these statistics for individual data

sets. Satisfactory calibration statistics show that calibration algorithms identified a common spectral response to changes in percentage of a target plant between independent trials. The reason for the poor calibration of alfalfa and grass in the juniper calibration feeding trial is not clear.

Table 1. Comparison of calibration statistics with reciprocal validation statistics from independent feeding trials.

	r^2			Slope			SECV	RMSEP	
Plant	Calib[1]	Best[2]	Worst[3]	Calib	Best	Worst	Calib	Best	Worst
Leafy spurge	0.92	0.87	0.66	0.91	0.59	0.66	5.3	16.6	22.6
Knapweed	0.96	0.64	0.23	0.96	0.39	0.98	5.6	14.7	17.5
Sagebrush	0.97	0.94	0.61	0.97	0.67	1.14	1.8	9.0	7.3
Alfalfa	0.98	0.17	0.35	0.98	0.39	0.11	3.8	12.7	27.4
Grass	0.98	0.52	0.29	0.98	0.94	-0.11	4.3	5.5	34.9
Juniper	0.93	0.75	0.65	0.93	0.58	0.75	5.0	14.6	11.3
Alfalfa	0.86	0.57	0.06	0.86	1.36	0.28	15.3	44.2	75.2
Grass	0.89	0.39	0.14	0.89	1.02	-0.21	15.7	35.0	64.0

[1]Calib are the calibration statistics resulting from combining two independent feeding trials. The RMSEP is based on independent validation.

[2] For the two independent calibration data sets that were used reciprocally to calibrate and validate each other, Best refers to the validation statistics for the calibration equation that had the highest r^2 for the independent validation of the target plant.

[3] For the two independent calibration data sets that were used reciprocally to calibrate and validate each other, Worst refers to the validation statistics for the calibration equation that had the lowest r^2 for the independent validation of the target plant.

Across all calibrations the mean r^2 was 0.94; however, for the best independent validations of the target plants the mean r^2 = 0.80 and for the worst set of independent validations of target plants the mean r^2 = 0.54. Based on recommendations by Williams (2001), the best r^2 were adequate for quality control and the worst r^2 (except for knapweed) were adequate for screening. The deviation of slopes from 1.0 (i.e., <0.80 or >1.15) for both the best and the worst independent validations were large enough to indicate that calibrations may be very sample sensitive (Williams 2001). Furthermore, the deviation of slopes from 1.0 was greater for the best independent validation indicating that precision and accuracy of calibrations were not related. Independent validation of grass and alfalfa backgrounds was much worse than for the target plants. This shows the importance of properly structured calibration diets that provide a diversity of backgrounds for the different percentages of plants for which calibrations are to be developed. This was generally the case for the target plants but not for the background forages in these data sets.

Poor results of independent validations reflect the fact that the optimal solution for a set of fecal diet pairs from a trial may differ from another independent feeding trial. This is demonstrated in these data sets by the generally low correlation between the coefficients of MPLS calibration equations of reciprocal data sets, which ranged from r^2 = 0.72 (sagebrush) to r^2 = 0.42 (knapweed). A single feeding trial with one source of target and background forages will not contain sufficient variation to predict samples from a different population. Independent validation for N and digestibility in Chapter 4 shows that combining two different calibration sets improves validations. Because using a calibration from one set of samples to determine the botanical composition of

another set of samples is problematic, the remainder of this chapter will examine the implications and potential solutions to this dilemma.

A potential solution for developing and monitoring F.NIRS calibrations would be to use the microhistological method (Sparks and Malechek, 1968) as the standard laboratory method for determining botanical composition of diets. Comparisons were done with a forb (leafy spurge) and a woody plant (juniper, Table 2).

Table 2. Comparison of validation statistics of microhistological estimates of leafy spurge and juniper in fecal samples with internal and independent F.NIRS determinations.

	Leafy Spurge				Juniper			
	Actual	Micro[1]	Inter.[2]	Indep.[3]	Actual	Micro	Inter.	Indep.
r^2		0.30	0.91	0.73		0.61	0.94	0.73
Slope		1.0	1.0	0.8		0.8	1.0	0.8
RMSEP		20.7	7.1	21.6		10.4	5.5	12.8
RMSErep[4]		13.4	9.9	11.5		8.4	1.5	2.8
Mean[5]	46.3	38.8	45.3	62.5	25.0	21.1	20.8	15.3
Difference[6]	72.5	22.7	64.5	65.3	30.0	21.8	27.3	26.4

[1] Microhistological validation statistics.
[2] Internal validation statistics based on calibrations from samples from the same feeding but that were not included in the microhistological analysis.
[3] Independent validation statistics based on calibration from a separate feeding trial.
[4] RMSErep was calculated as the square root of the mean of the squared differences between two replicate animals with the same level of the target plant in their diet.
[5] Mean of all samples in the validation data set.
[6] Difference between with highest and lowest percentage of the target plant in the validation diet as determined by the different methods.

Microhistological estimates of percentage of leafy spurge or juniper in the diet were similar to F.NIRS in terms of accuracy (slope) but not in terms of precision (r^2). The slope of estimated to actual composition by the microhistological analysis was similar to both internal and independent F.NIRS determinations. Accuracy of microhistological and internal determinations in terms of the overall estimated mean by these two methods was similar and better than the independent calibration estimate, but both F.NIRS determinations estimated the difference between the highest and lowest diets with greater accuracy than the microhistological technique. Precision was lower for the microhistological method than for either the internal or independent F.NIRS determination. The RMSEP, which contains errors caused by lack of accuracy and precision, was similar between microhistological and independent F.NIRS estimates. Root mean square error of replicate samples (RMSErep) was greater for microhistological than for either of the F.NIRS determinations. Not surprising, microhistological estimates of juniper (woody plant) for which twice as many slides were read were more accurate and precise than estimates for leafy spurge a (highly digestible forb). Determinations by internal and independent F.NIRS equations were not affected greatly by the species of plant that was predicted.

Naes et al. (2002) suggest that if the reference method provides an unbiased estimate but imprecise measurement of the true value, that reference method can be used to develop calibrations with an error of $\sigma/2$ where σ = the standard deviation of replicate determinations. However, calibrations

using microhistological values as constituent data were not usable. The ability of the microhistological method to estimate accurately the mean of a population indicates that if accuracy of F.NIRS determinations is doubtful, the microhistological method could be used to estimate the actual mean of the population. However, the microhistological procedure would not be as useful as F.NIRS for estimating treatment differences. If an independent verification of overall percentage of a target species was desired, a cost-effective approach would be to use F.NIRS to determine individual animal diets and the microhistological method on a composite of all samples to estimate the average composition of diets.

Including samples that did not contain the target plant (i.e., zero fecals) in the calibration set improved the r^2 for the worst of the pair of reciprocal calibrations but not for the best reciprocal calibration (Table 3). The greatest improvement in validation statistics was a reduction by about half in the RMSEP of both best and worst reciprocal validations as a result of adding zero fecals to the calibration set. Zero fecals can be obtained by removing all of the target plant from an area and then allowing animals to graze the area for 5 days before collecting fecal samples. Adding zero fecals to a calibration data set appears to be a practical way to increase the accuracy of F.NIRS predictions. Adding additional zero fecals not related to the validation population was investigated, but this resulted in worse predictions and is not recommended.

Table 3. The effect of adding zero fecals (i.e., diets that do not contain the target plant) on validation statistics. Comparisons are between calibration equations either with (+ 0) or without the zero fecals from the reciprocal data set. Validation sets are the same for both calibrations.

	r^2		Slope		RMSE	
Plant	Best	Worst	Best	Worst	Best	Worst
Knapweed	0.58	0.31	0.41	1.52	15.5	15.0
Knapweed + 0	0.49	0.47	0.37	0.97	23.5	8.9
Sagebrush	0.92	0.64	0.68	1.18	9.5	6.3
Sagebrush + 0	0.87	0.92	1.10	0.74	3.9	4.8
Juniper	0.72	0.61	0.55	0.75	14.1	11.7
Juniper + 0	0.78	0.87	0.70	0.84	6.5	7.6

The effect of number of levels of a target species and number of levels of background forages in the calibration diet is shown in Table 4. In this study, background diets, to which sagebrush was added to obtain the specified composition of sagebrush, consisted of alfalfa hay, grass hay and four mixtures of these hays in 20 percentage unit increments from 20:80 to 80:20 alfalfa:grass, respectively. These results show that maximizing the diversity of background forages is the most important factor affecting the determination of independent samples. Elimination of intermediate levels of sagebrush between the highest and lowest, while keeping all different background diets, had little effect on the validation statistics even though the number of calibration samples was reduced by about half. Furthermore, comparisons of reduced calibration data sets both with 23 calibration samples consisting of alfalfa or grass hays compared to 40:60 and 60:40 mixtures of these two hays showed that

using two unique background diets gave better predictions. This finding regarding maximizing diversity of background forages is further validated by the reciprocal validations of the two juniper trials, where the calibration that validated the best was one that contained eight different background forages. It also confirms the observation (Table 1) that validation of background forages had less precision and accuracy than the target species because the background forages varied across a single target plant.

Table 4. Effect of number of levels of target plant (sagebrush) and differences in background diets in calibration data set on validation statistics for independent validation statistics.

Calibration Samples			Validation Statistics		
% Sagebrush	Background	n	r^2	Slope	RMSEP
0, 4, 8, 12, 16, 24	All[1]	137	0.94	0.79	5.6
0, 4, 24	All	69	0.91	0.75	7.6
0, 4, 24	Mixed[2]	23	0.78	0.62	17.2
0, 4, 24	Pure[3]	23	0.82	0.72	9.5

[1] All background diets include alfalfa hay, grass hay and four mixtures of these hays in 20 percentage unit increments from 20:80 to 80:20 alfalfa:grass, respectively.
[2] Mixed backgrounds are diets with mixtures of the two hays.
[3] Pure backgrounds are diets with either alfalfa or grass hay.

Validation of an independent juniper calibration with diets containing a variety of background forages showed that if r^2 was calculated across all background diets the precision was reduced (Table 5). When validation statistics were calculated within individual background forages, the precision increased markedly with 62% of the background forages having an $r^2 > 0.9$. The RMSEP was improved in one-half of the determinations with only one background forage. For background forages where RMSEP was not improved, it generally was because the slope deviated significantly from one. In most applications of F.NIRS to determine botanical composition of diets, the interest will be in testing for treatment effects of animals grazing common or similar pastures. These results indicate that in such applications the F.NIRS determination may be more precise than indicated by validation r^2, particularly if it is calculated across a variety of background forages, because of a presumably relatively uniform background diet by animals on the same pasture.

Table 5. The effect of calculating validation statistics within the same background diet compared to calculations across all backgrounds diets when using an independent calibration equation for juniper.

Background	r^2	Slope	RMSEP
All Backgrounds	0.57	0.99	14.0
Wheat Hay	0.76	0.85	9.2
Ryegrass	0.79	1.87	27.9
Forbs	0.79	0.59	15.7
Bermudagrass	0.94	1.18	17.8
Alfalfa	0.96	0.77	5.3
Peanut Hay	0.97	0.89	13.6
Haygrazer	0.99	0.93	6.2
All hays + 10% browse	0.97	0.83	5.3

The potential for introducing bias or reducing precision as a result of developing calibrations based on dried samples of the target plant to determine percentage of that plant consumed by grazing animals was tested for sagebrush and spotted knapweed. Independent calibrations were developed for both species using air-dried forages to determine the composition of diets of sheep fed known amounts of the forages as either freshly harvested (knapweed) or frozen (sagebrush) and compared to similar diets in the same feeding trial where the target plant was air-dried. F.NIRS determined amounts of the target forage did not differ between fresh and dry ($P \geq 0.28$) nor was there an interaction between state and levels of the target plant fed ($P \geq 0.14$). These calibrations were able to detect differences in the percentage of the target plants in the diets ($P < 0.001$) even though the differences between the F.NIRS percentages were smaller than the actual differences in the percentages fed. Results of these two calibrations, one using a woody plant high in volatile oils and the other, a forb containing aversive alkaloids, show that air-drying these plants before mixing diets for a calibration trial does not affect the ability of F.NIRS to detect differences in the percentage of the plant when consumed fresh and that F.NIRS-determined percentages do not differ between fresh and dried plants.

The length of time required for F.NIRS determinations to equilibrate and remain constant is shown in Figure 1.

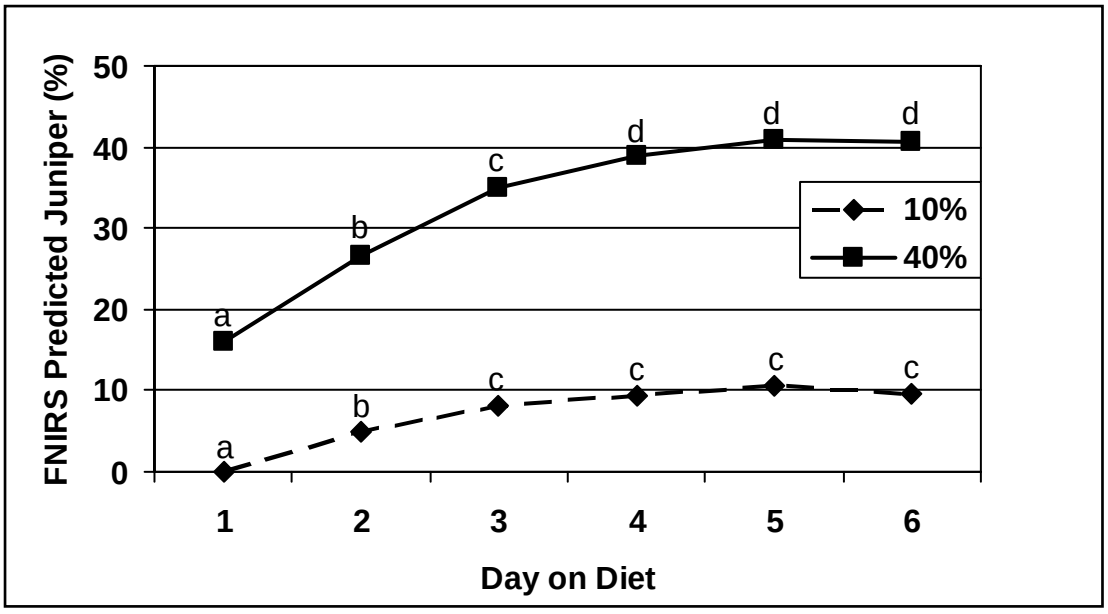

Figure 1. Daily change in F.NIRS determinations of percentage of juniper in the diet of goats going from either 0 to 10% juniper or 10 to 40% juniper. Within a level, means with different letters were significantly different ($P < 0.05$).

In this experiment, goats were sequentially fed diets with 0, 10 or 40% juniper and a variety of backgrounds for 8 days at each level beginning at 0% and progressing to 40% juniper. Calibration equations were based on the last 2 days at a given level and were used to predict percentage of juniper for the first 6 days on the diet. The time required appeared to depend upon the amount of change in a diet. When going

from 0 to 10% juniper, F.NIRS determinations did not change after the third day ($P < 0.05$), but when the diet changed from 10 to 40% juniper, F.NIRS determinations did not stabilize until the fourth day. Mean digestive tract residence time in goats is 36 – 60 hr (Castle 1956). The slightly longer equilibration period as well as increased equilibration period associated with larger changes in the diet indicate that changes in fecal spectra associated with diets differing in botanical composition are not due solely to changes in undigested feed but may also be caused by differences such as changes in microbial populations that may lag behind mean residence time.

The effects of breed, sex, and age were investigated using a calibration developed from previous juniper feeding trials to predict the percentage of juniper in the diet of goats that were fed a pelleted diet containing either 0 or 14% juniper (Walker et al. 2007). The difference in level of juniper in the diet was readily detected ($P < 0.001$), and F.NIRS difference between the two diets was near the actual difference between the actual diets, but the percentage of juniper predicted was about twice as high as the amounts fed (Table 6). F.NIRS determinations of percentage of juniper in the diet of male goats was about 50% higher ($P < 0.005$) than females even though both sexes were on the same diet. The effects of breed and age equations were not different.

Table 6. The effects of breed, sex and age on F.NIRS-determined mean percentage of juniper (SD) and probability of difference in the diet of goats fed a complete pelleted diet containing either 0 or 14% juniper.

	F.NIRS Percentage Juniper Determinations[1]	
Breed & sex comparison		
% Juniper in diet	$P < 0.001$	
0	9.3	(7.7)
14	22.4	(4.6)
Breed	$P = 0.492$	
Angora	15.3	(11.4)
Meat	16.4	(6.3)
Sex	$P = 0.004$	
Intact male	18.4[a]	(6.2)
Castrated male	17.2[a]	(7.5)
Female	11.9[b]	(11.8)
Angora age & gender comparison		
% Juniper in diet	$P < 0.001$	
0	4.2	(9.9)
14	22.3	(5.5)
Age	$P = 0.383$	
Adult	14.1	(12.9)
Kid	12.3	(11.4)
Gender	$P = 0.003$	
Intact male	16.5	(10.0)
Female	10.0	(13.3)

[1] Independent modified partial least squares calibration equations were developed using data from feeding trials conducted in 1999, 2002, and zero fecals from goats grazing pastures with no juniper.
[a,b] Within-variable means of gender group, means without a common superscript differ ($P < 0.05$).

Treatment differences in SD (Table 6) provide insight into limitations of the calibration equations. Comparison in the adult data set of the standard deviations of Angora to meat goat and female to male or castrated male also showed over a 70% greater SD for Angora and female compared to the other classifications (*P = 0.04*). Furthermore, on average, SD for determinations in the Angora data set were greater than ones from the adult data set. We assume that the larger SD for Angora female adults and kids was because independent calibrations were developed primarily with fecal spectra from meat goat wethers. Thus animals used to develop calibrations should be of the same sex as the animals the calibrations are used on or comparisons should not be made between sexes.

CONCLUSIONS

Near-infrared spectra from feces can be used to develop calibration equations for predicting the botanical composition of the diet that animals are consuming. However, precision and accuracy are reduced greatly when calibrations from one feeding trial are validated with an independent feeding trial. Microhistological analysis of fecal samples cannot be used to monitor equations because this procedure has lower precision than the independent validations. Because of the difficulty of monitoring F.NIRS calibrations for determining botanical composition, probably the best that can be expected is that F.NIRS determinations should be considered interval scale measurements. An interval scale of measurement means that treatments can be ranked and that the difference between treatments has meaning and are equal across the range of measurements, but there is not a true zero point. Thus, it is appropriate to say that the difference between treatment A and C is twice as large as the difference between treatment A and B. But it would not be appropriate to say that treatment A is twice as large as treatment C.

Several principles that can increase the robustness of calibrations were demonstrated. Feeding trials to produce fecal diet pairs for F.NIRS calibrations should use as much variety in the background forages as possible. Several diets, each with a single background forage rather than several diets with different combinations of background forages, will provide more robust calibrations. The number of levels of the target plant in the diet does not need to be large. Three levels of a target plant consisting of the highest percentage that is expected to occur in the diet, a low level (e.g., 10%) and an intermediate level, plus the background forages with none of the target plant should be sufficient. These levels should be replicated with as many distinct background forages as possible. Fecal samples from animals grazing the same type of vegetation as the animals that are being predicted but with none of the target plant (zero fecals) can be included in the calibration to increase accuracy. Diets should be fed for a minimum of 4 days before collecting fecal samples for scanning. Calibrations developed using air-dried forages, even forages that contain volatile compounds, can predict diets developed with fresh plant material. Sex of the animal appears to affect determinations, and comparisons should not be made between different sexes unless the animals used in the calibration trials reflect the sexes of the animals being compared.

F.NIRS to determine botanical composition of diets has been used to identify high and low consumer groups of chemically defended plants that differ in their consumption of these plants in subsequent controlled studies (Campbell et

al. 2007; Fraker-Marble et al. 2007). This technique also has been used to determine consumption of sagebrush by sheep (Snowder et al. 2001) and juniper by goats (Waldron et al. 2009) so that heritability for this trait could be calculated.

Botanical composition of herbivore diets is an important attribute that affects composition and succession of plant communities and the competitive interactions between sympatric herbivores. As such, diet composition has been studied widely, but these studies are often limited because of the techniques available for determining the botanical composition of diets. F.NIRS to determine botanical composition offers a more precise alternative to the microhistological procedure and costs about 10% as much as microhistological analysis. This makes the F.NIRS procedure a viable alternative that is particularly useful for comparing treatments or individual animals.

ACKNOWLEDGEMENTS

This research was supported by Research Grant No. IS-3555-04 from BARD, the United States - Israel Binational Agricultural Research and Development Fund, and by Texas Food and Fibers Commission Project No. b-04-05-02. ARO Publication no. 136/2005.

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Chapter 6. Fecal NIRS with Bite Counts: A Methodology to Determine the Botanical and Chemical Composition of Diets Consumed by Goats in a Mediterranean Shrubland

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Objectives: To describe a method for using bite-count and hand-plucked forage samples that mimic bites to develop F.NIRS diet-fecal pairs for calibrations.

Key Points

- A method combining bite-count on target animals and F.NIRS was developed.
- Direct continuous observation of target goats recorded botanical composition of diets and hand-plucked forage representing bites was used to determine chemical and botanical composition.
- Fecal samples collected on the second and third day following observations were mixed to develop diet-fecal pairs with the hand plucked diet.
- Botanical composition calibrations of 2 browse species and total herbaceous forage were reasonable with R^2 between 0.77 – 0.89 and SECV between 5.6 – 7.8% DM.
- Calibrations for CP, NDF, in vitro DM digestibility would reliably predict diets of goat browsing in this environment. Calibrations for PEG binding tannins can only be used for screening purposes.
- Calibrations for DM intake were poor.

INTRODUCTION

Brush encroachment is a problem worldwide, and targeted grazing by goats might be an ecologically sound approach to this problem (Campbell and Taylor 2006). However, better knowledge of their feeding selectivity and their ability to thrive in encroached areas is required in order to devise viable production systems.

Direct observation could provide precise and accurate estimates of diet selection (Agreil and Meuret 2004), but the method is too time-consuming for construction of a sufficiently large database to clarify the effects of season, breed, and location on the propensity of goats to consume browse species.

Fecal near-infrared reflectance spectroscopy (F.NIRS) can determine both the chemical (Leite and Stuth 1995) and the botanical (Landau et al. 2004a) composition of goat diets, but the methodology must be applied with care. Walker et al. (2002) showed that the application of calibration equations developed in one feeding trial to fecal samples gathered in another (*i.e*., external validation) yielded predictions of low accuracy. Indeed, Coleman et al. (1995) stated that NIRS equations cannot be extrapolated beyond the conditions represented in calibration samples, and Landau *et al*. (2005) demonstrated that similar structures of calibration and validation populations are a prerequisite for successful external validation of F.NIRS equations. Because of the very high workload imposed by direct observations, we devised a methodology where a subsample (focal goats) of a flock was observed. These observations serve as reference values for F.NIRS calibrations that can be used to predict the diets of other goats grazing simultaneously (resident animals). In other words, the calibrations presented here were planned to be used only in the specific region and with the specific animals of this project.

The present study describes the development of F.NIRS calibrations for botanical and nutritional composition of diets consumed by free grazing goats in the Carmel Heights of Israel. The reference values for diet-fecal pairs were determined with a bite-count methodology of observed animals.

MATERIALS and METHODS

Study Area

The study was conducted at the south of the Mount Carmel ridge, Israel (32°25' N, 34°52' E), which is characterized by an average yearly rainfall of 600 mm and a 180-day rainy season from October to April. The ecosystem is a disturbed Mediterranean woodland (garrigue), characterized by steep, rocky slopes with sparse patches of shallow soil. The vegetation is dominated by low trees (mainly *Phillyrea latifolia* L.) and tall shrubs (mastic tree, *Pistacia lentiscus* L. and *Calicotome villosa* Poir. Link) that form 2 to 3 m high coppices around islets that sometimes are covered with climbing *Rubia tenuifolia* D' Urv., *Clematis cirrhosa* L. and *Smilax aspera* L. Isolated common (*Quercus calliprinos* also named *Q. coccifera* Webb) and Tabor (*Quercus ithaburensis* Decne) oak trees, as well as carob trees (*Ceratonia siliqua* L.) and buckthorn (*Rhamnus lycioides* L.) trees can be found also. Occasional bushes of *Ephedra foemina* Forskk., *Asparagus stipularis* Forskk., and *Sarcopoterium spinosum* L. Spach grow between the coppices. From January to mid-May, green annual herbaceous vegetation covers the soil patches.

Five fenced 0.1-ha plots and four unfenced plots differing in aspect, slope, and botanical cover were used. Over the course of four seasons – spring, summer and fall 2004, and spring 2005 – foraging was rotated among the plots according to vegetation availability.

Animals and Management

In the spring and fall of 2004, the flock consisted of adult Damascus goats (n = 12). In the summer of 2004, these were culled and replaced with Damascus (n = 9), Boer (n = 9), and Mamber (n = 9) yearlings, managed as three separate groups. The goats were kept according to ICACG (Israel Council on Animal Care Guidelines – 1994). The groups were led out to forage in the mornings and were housed at night in a dirt-floored and roofed building. During fall of 2004 and spring of 2005, foraging was rotated among seven 0.1-ha fenced plots and an unfenced area according to vegetation availability. The animals were shepherded only in the unfenced area. Adult and yearling does received a daily ration of 90 and 138 g DM, respectively,of a commercial concentrate (Ambar Feed Mills Ltd.,Hadera, Israel) containing 18% CP, on a DM basis.

Collection of Dietary Data for Calibration. The dietary data required for the calibration of the F.NIRS procedure were collected in two stages. The first stage was direct and continuous observations of individual animals to determine the number of bites removed for each plant species and bite-type category (defined below). The second stage was collection of representative samples of each species and bite-type category for the determination of their mass and nutrient composition.

Observations on Goats and Observation Data Processing. Observations (n = 45) encompassed diets selected by adult Damascus does and yearling Boer, Mamber, and Damascus goats. Respective numbers of observations were 10, 11, 12, and 12. Goats were observed in five plots in four seasons (*i.e.*, spring, summer, and fall of 2004, and spring of 2005). Each observation comprised two consecutive days of observation on the same animal. The distribution of observations among goat breeds and seasons is presented in Table 1.

Table 1. Mean BW (kg, ± SEM) of the goats examined in the 45 observations, according to year, season and breed.

Year	Season	Breed	Age	n	Live weight (kg)
2004	spring	Damascus	adults	3	53.5 ± 1.3
	summer	Damascus	adults	7	51.2 ± 1.0
	fall	Boer	yearling	4	20.9 ± 1.1
		Mamber	yearling	4	18.1 ± 0.7
		Damascus	yearling	5	31.8 ± 0.5
2005	spring	Boer	yearling	7*	31.6 ± 1.7
		Mamber	yearling	8*	26.7 ± 0.5
		Damascus	yearling	7*	36.4 ± 0.7

*Not all different individuals.

Observations of foraging behavior were initiated after a 5-day period of acclimation to a new plot and always encompassed the entire day's foraging of the observed animal. Observations started between 0630 and 1040 (average 0800) and terminated between 1030 and 1440. The duration of an observation day ranged from 213 to 300 min (average 242 min), with 85% of observations lasting between 235 and 245 min. A complete observation (*i.e.*, pair of observation days) was double this length.

The observers were T. Glasser (n = 23), H. Muklada (n = 18), and a postgraduate student (n = 4).

An effort was made to observe as many different animals as possible, but only 30 different goats eventually served as focal animals. Individual animals were not used for observations if the continuous presence of an observer at a distance of approximately 1 m interfered visibly with their normal foraging behavior. Observations were recorded with a voice-activated digital MP3 recorder. When a focal goat started to eat, the recorder was operated, time was automatically recorded, and the observer recorded a sequence of codes that combined species and bite-type category (small, medium or large, leaf, item or fruit). A few of the bite-type categories defined consumption units that were not bites in the usual sense of the term. For example, *Ephedra foemina* was consumed by severing a relatively long section of branch and then bringing it into the mouth by chewing; therefore, in summer 2004, each consumption unit of this species was recorded as the number of centimeters consumed. Table 2 shows the number of bite-type categories defined for each species in each year-season combination.

Table 2. The total number of bites recorded for each species in each year-season combination. Values are totals for all bite-type categories within a species.

Species	Spring 2004	Summer 2004	Fall 2004	Spring 2005	Total
True bites					
Phillyrea latifolia	2775	6745	20249	18525	48294
Rhamnus lycioides	765	2863	4123	29528	37279
Smilax aspera	302	2698	11818	11611	26429
Sarcopoterium spinosum	538	-	1694	21468	23700
Pistacia lentiscus	952	2085	6045	8263	17345
Rubia tenuifolia	248	2705	2291	10881	16125
Asparagus aphyllus	400	2472	6451	746	10069
Calicotome villosa	1722	-	287	5013	7022
Euphorbia sp.	-	-	-	2665	2665
Clematis cirrhosa	323	-	-	1898	2221
Asphodelus ramosus	91	70	231	1305	1697
Ceratonia siliqua	-	689	38	-	727
Prasium majus	-	-	-	720	720
Quercus calliprinos	-	340	-	-	340
Allium sp.	-	-	-	291	291
Pistacia palaestina	-	153	-	-	153
Tamus communis	-	-	-	140	140
Cyclamen persicum	-	-	-	124	124
Sinapis arvensis	-	-	-	68	68
Scabiosa prolifera	-	-	-	62	62
Eryngium creticum	-	-	-	61	61
Quercus ithaburensis	-	53			53
Unidentified	21	20			41
Olea Europaea	34	-	-	-	34
Other consumption units					
Ephedra foemina	-	17221	341	918	18480
Herbaceous (dry)	310	5417	1460	-	7187
Herbaceous (green)	84	-	-	2746	2830
Smilax aspera	-	-	165	-	165

To trim periods of silence from recorded electronic voice files (MP3) during the 4-hour observations, time-signal (every 30 s) files (wav) were created (Cool Edit Pro ver 2.0; Adobe Systems, Inc., San Jose, CA) and combined with the voice files. Silent periods were trimmed using Sonic Foundry (version 6.0, Sonic Foundry, Inc., Madison, WI). This procedure resulted in significantly shorter files (1 to 1.5 hours). The bite count and time data from the trimmed files were then keyed manually into an Excel spreadsheet. A total of 195,660 true bites and 27,921 consumption units (species-category combinations that were not true bites) were recorded.

Simulated Bites Collection. To estimate the goats' intake and the quality of the diet bite-like samples were clipped so that the sample collection combined species and bite-type categories, according to the recorded foraging behavior**.** The intake of herbaceous vegetation was evaluated by cutting "estimated mouthful" samples and intake of *E. foemina* by clipping phyllode (flattened leaf stalk functioning as a leaf) segments of various lengths. This resulted in a total of 17,555 bite-like samples, of which 4,188, 3,095, 5,072, and 5,200 samples were collected in spring, summer, and fall of 2004 and spring of 2005, respectively. The DM contents were assessed immediately after collection by drying the bite-like samples at 60°C for 48 hours in a forced-air oven. Higher temperatures could not be used because of the volatile components, especially phenolics, in browse foliage. Bite weights were then calculated by combining species and bite-type categories. Total species daily intakes were calculated as the weighted product of the number of bites and category bite-weights and summed into total intake for each 2-day observation.

Laboratory Analysis of Simulated Bites and Calculation of Nutrient Intakes. To obtain adequate amounts of material for laboratory analysis, bite-like samples were merged into 180 species and bite categories to yield 41, 40, 40, and 59 samples for spring, summer, and fall of 2004 and spring of 2005, respectively. The samples were then ground to pass a 1-mm sieve. The *in vitro* digestibility of dry matter (IVDMD) was evaluated according to Tilley and Terry (1963). Crude protein (CP) was assayed by using an automated Kjeldahl method (976.05; AOAC, 1990); NDF and ADF were assayed according to Goering and Van Soest (1970). The content of PEG-binding tannins (PEG-b-T) was determined by NIRS without extraction, according to Landau et al. (2004b).

The intakes of CP, NDF, ADF, *in vitro* digestible DM and PEG-b-T were calculated from the sum of bites per species and category, multiplied by estimated bite weight, expressed on DM basis. The percentage of dietary nutrients were calculated as nutrient intakes divided by DM intake. Nutrient intake and DM intake included both pasture and concentrate intakes.

Collection of Feces for Calibration. The goats stayed on the same plot for at least 3 days after an observation day. On the second and third days, feces were grab-collected from the anus of observed goats in the morning, at midday, and in the evening. A composite sample for all the times and both days for each animal was dried at 60°C in a ventilated oven for 48 h and ground to pass a 1-mm sieve. At 0600 on days of fecal sampling, the animals (without feed or water restriction) were weighed with a model Merav 2002 electronic balance with an

accuracy of ± 10 g (Shekel Balances, Rosh Ha-Ayin, Israel).

NIRS Procedures

Preparation of Fecal Samples. Fecal samples were re-dried at 60°C for 1 hour, allowed to equilibrate in a desiccator at ambient temperature for 1 hour, packed into sample cells with a near-infrared-transparent quartz cover lens, and scanned at wavelengths from 1104 to 2492 nm in 2-nm increments with a Foss NIRSystems (Hoganas, Sweden) model 5000 NIR reflectance monochromator spectrometer in order to collect NIR spectra as log (1/R) where R = reflectance.

NIRS Calibration Equation Development. Before calibration equations were developed, raw spectral data were transformed with the Standard Normal Variance (SNV) and detrend procedures to remove the non-linearity caused by light scattering (Barnes et al. 1989). Derivative and smoothing mathematical treatments to enhance spectral differences where 2, 6, 6, 1(for D,G,S1,S2; Chapter 1 this volume; ISI 1999). Spectral outliers were searched for by using the Mahalanobis distance between each of the fecal samples and the mean spectrum of the calibration dataset (Shenk and Westerhaus 1991). A modified partial least squares regression (Martens and Naes 1987) was used to develop calibration equations in which stored NIRS spectra from fecal samples were the independent variables and nutritional attributes were the dependent reference data.

Calibration precision was evaluated according to the multiple coefficient of determination (R^2), (*i.e.*, the proportion of variability in the reference data accounted for by the regression equation). The standard error of calibration (SEC) defined the variability in the differences between predicted and reference values. The calibration accuracy was evaluated by cross-validation and expressed as the standard error of cross-validation (SECV). The SECV is the average root mean square difference between predicted and reference values when the equation is calculated and applied sequentially to subsets (of which there were 4 in the present study) of data from the calibration data set. The SECV procedure may give over-optimistic results, especially if data are replicated, but is justified in situations where the calibration samples are selected randomly from a natural population (Naes et al. 2002). We did not attempt to carry out external validation because we did not intend to use the F.NIRS equations beyond the site where they were developed. When regressions of observed *vs*. predicted values were examined, the closeness of slopes to unity and of intercepts (bias) to zero served as criteria of the usefulness of the calibrations.

RESULTS

Reference Value Database

Table 2 shows the partition according to species and season of the 195,660 individual true bites and 27,921 consumption units (species-category combinations that were not true bites) recorded. Figure 1 shows the variation in the bite weights obtained for each species that was attributed to year-season combination and bite-type category in the 180 merged samples subjected to laboratory analysis. Fifty-three percent of true bites and consumption units weighed less than 0.25 g, 16% weighed 0.25 to 0.5 g, 15% weighed 0.5 to 1.0 g, and 16% weighed more than 1.0 g, on a DM basis. The largest bite weights were noted for *P. lentiscus* (5.2 g, spring of 2004), *Olea europaea* (4.8 g, spring of 2004), *P. latifolia* (4.4 g, fall of 2004) and *Q. calliprinos* (4.3 g, summer of 2004).

After total species daily intakes were calculated, it appeared that two observations from fall of 2004 recorded extremely low intakes (349 and 506 $g.d^{-1}$, compared with an average of 1086 ± 45 $g.d^{-1}$ for the whole dataset), which strongly suggested impaired health. Their associated F.NIRS reflectances in a number of wavelengths (*i.e.*, around 1900 nm [C=O stretch in COH2], 1920 nm [C=O stretch in CONH] and 1940 nm [water]) were atypical, and the spectra featured the highest Mahalanobis values in the dataset. Because the objective was to devise a dietary predictive methodology for healthy animals, these two observations were discarded from the dataset used for fecal NIRS calibrations.

The wide variety of nutrient contents in bite-like samples is depicted in Figure 2. The content of CP varied between 3.5 and 23.7% of DM, and that of PEG-binding tannins between 0 and 27% of DM. Minimal and maximal ranges for dietary percentages on DM basis were 5.6 to 13.0% CP, 38.5 to 56.7% NDF, 23.6 to 36.4% ADF, 32.3 to 67.5% *in vitro* DM digestibility, and 3.6 to 11.6 % PEG-binding tannins.

Fecal NIRS Calibrations

Examination of the Mahalanobis spectral distances from the mean fecal spectra showed that 65% of the standardized H values were below 1, 31% were between 1 and 2, and the remainder between 2 and 3. In other words, no spectral outliers (H > 3 from individual spectra to the population centroid; Shenk and Westerhaus 1991) were found in the fecal spectra used for calibration.

Diet-fecal pairs from all years and seasons were combined, and calibrations for dietary percentages of botanical and nutritional constituents were developed. Because *P. latifolia* was the main tree, *P. lentiscus* was the main encroaching brush species, and herbaceous vegetation was important for nutritional reasons, calibrations are given only for these botanical entities, in addition to nutritional constituents.

The performance of the F.NIRS calibrations is summarized in Table 3. The R^2 values for *P. latifolia,* herbaceous, and *P. lentiscus* were 0.89, 0.85, and 0.77, respectively, with respective SECV values of 6.3, 7.8, and 5.6% of DM, and averages of 17.6, 22.3 and 8.7% of ingested DM. The slope of the relationship between the bite-count-estimated and fecal NIRS-predicted values for *P. latifolia* was 0.90, (i.e., different from [$P < 0.05$] but still reasonably close to unity, and the intercept did not differ from zero [$P = 0.10$]).

Within the calibrations for nutritional attributes, the lowest R^2 value (0.74) was found for PEG-binding tannins, all the others being close to 0.90. The accuracy of the PEG-binding tannin calibration (SECV of 0.88 for an attribute average of 4.83% of ingested DM) was also the lowest. The SECV values of the dietary percentages of CP, NDF, ADF, and *in vitro* DM digestibility were low, relative to average values for these attributes, but only the CP and *in vitro* DM digestibility calibrations could be considered as totally unbiased, with slopes not significantly different from unity and intercepts not significantly different from zero.

Compared to calibrations of the percentage of dietary constituents, the R^2 value for the calibration of total daily nutrient intakes (not shown) were low: 0.18 for DM; 0.59 for CP; 0.13 for NDF; 0.52 for in vitro DM digestibility; 0.50 for PEG-binding tannins; and 0.20 for ADF. As to botanical intakes, the R^2 value for the rates of intake of both herbaceous and *P. latifolia*

was higher than the R^2 for *P. lentiscus* (0.80 and 0.65, respectively). The rates of intake of herbaceous and *P. latifolia* (*i.e.*, 157 and 122 $g.d^{-1}$) were predicted with respective SECV values of 71 and 64 $g.d^{-1}$. The slopes of actual *vs*. predicted values of herbaceous and *P. latifolia* intakes were close to 0.80; they differed from unity ($P < 0.01$), and had non-zero (27 $g.d^{-1}$; $P < 0.05$) intercepts.

Table 3. Modified partial least squares calibration statistics for percentage dietary botanical and nutrient composition in the diets of free grazing goats. Nutrient composition includes nutrients supplied by concentrates. Calibrations contained 43 fecal-diet pairs and spectral data were pretreated to remove scatter effects using standard normal deviate and detrend with 2, 6, 6, 2 derivative math treatments.

	Diet		Calibration				
Constituent	Mean	SD	SEC	R^2	SECV	slope	Intercept
Botanical							
Herbaceous	22.3	12.5	4.9	0.85	7.8	0.87	2.8
P. latifolia	17.6	13.1	4.3	0.89	6.3	0.90	2.0
P. lentiscus	8.7	9.8	4.6	0.77	5.6	0.78	2.1
Nutritional							
CP	11.0	2.29	0.62	0.93	0.87	0.95	0.70
NDF	41.9	3.74	1.30	0.88	2.14	0.75	10.0
ADF	25.3	2.88	0.98	0.89	1.69	0.90	2.4
IVDMD	56.4	10.6	3.16	0.91	4.27	0.92	4.4
PEG-b-T	4.8	1.44	0.73	0.74	0.88	0.75	1.2

DISCUSSION

Fecal NIRS calibrations for dietary chemical composition of free-grazing goats have been reported before (Leite and Stuth 1995; Landau et al. 2004a), but here we address F.NIRS with reconstituted diets based on bite counts and on the simulated bite method for reference values. Compared with the use of fistulated animals (Leite and Stuth 1995), the bite count methodology has three advantages: 1) information is obtained for entire grazing days; 2) the same animal is used for diet estimation and fecal sampling; and 3) diets selected by fistulated animals may be different from those of unfistulated residents (Coates et al. 1987). The esophageal extrusa samples collected by Leite and Stuth (1995) consisted of the diet actually consumed by animals, whereas in this study, the diets were simulated with bite counting. Bite counting has inherent risk of errors, particularly in the estimation of bite weights for species with low bite-weights (Figure 1).

In the present study, 10 species/groupings accounted for just over 90% of the estimated total intake. They were, in descending order of total consumption: *P. latifolia*, green herbaceous vegetation, *P. lentiscus*, *S. aspera*, *R. lycioides*, *R. tenuifolia*, *S. spinosum*, dry herbaceous vegetation, and *E. foemina*. Because bites can be counted accurately by an experienced observer, accurate estimation of the bite weight for these species is critical for successful estimation of intake. There are limited published data regarding the bite weight of goats foraging in Mediterranean shrubland, and bite weights, which represent an intermediate stage in intake calculations, are rarely published. Bite weights reported in the present study are similar to those

reported by Aharon et al. (2007) and Z. Henkin (ARO, Newe Yaar, Israel, personal communication) for *R. lycioides*, *S. aspera*, *S. spinosum*, and herbaceous vegetation, and those reported by Decandia et al. (2000) and M. Decandia (IZCS, Bonassai, Italy, personal communication) for *P. lentiscus* and *P. latifolia*. Intake values for the above species in the present study were comparable with those reported by Kababya et al. (1998) and Decandia et al. (2000).

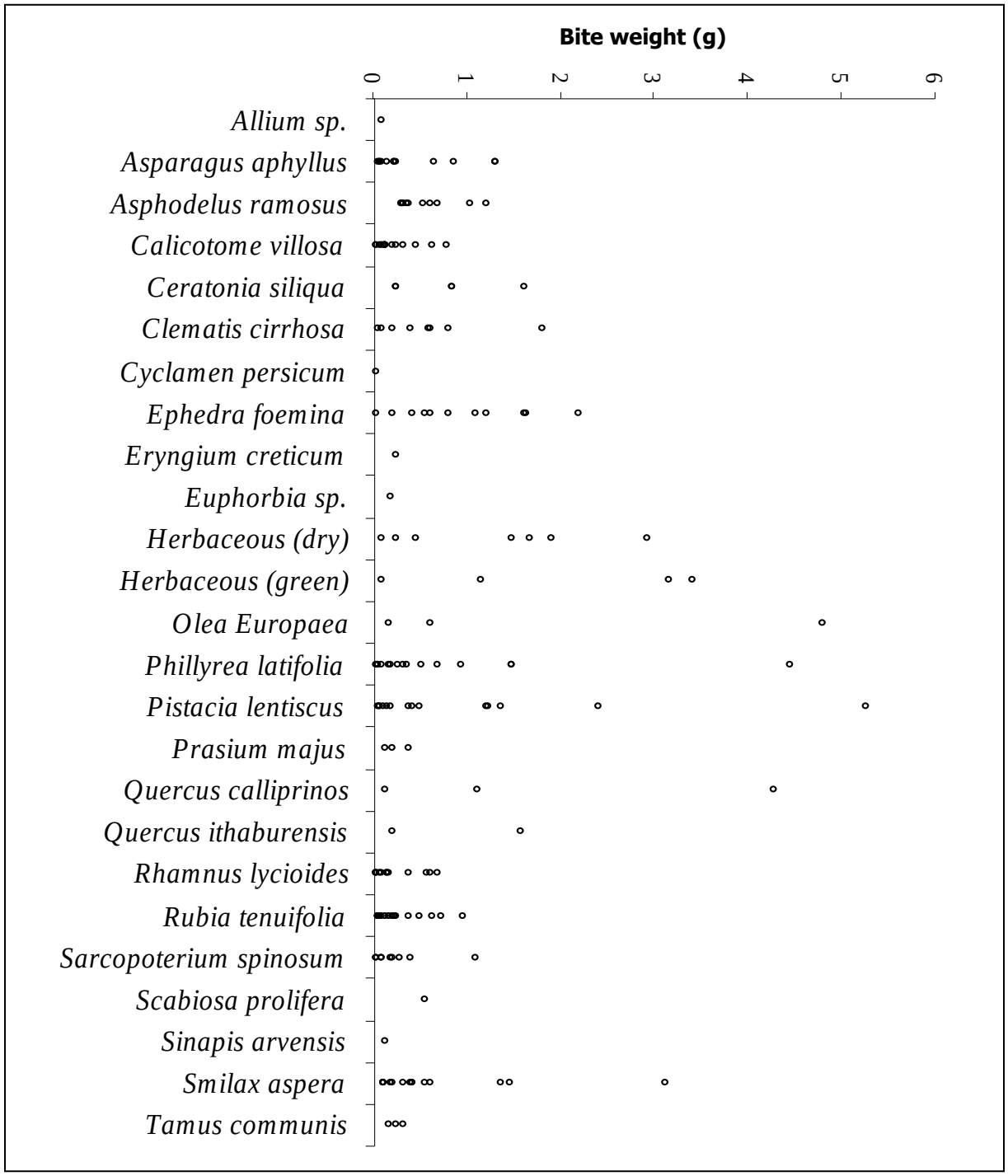

Figure 1. Bite weight (g) values obtained for each species for the various bite-type categories and year-season combinations (n = 180). Y-axis: Bite weight (g). Reused by permission of the American Society of Animal Science © 2008.

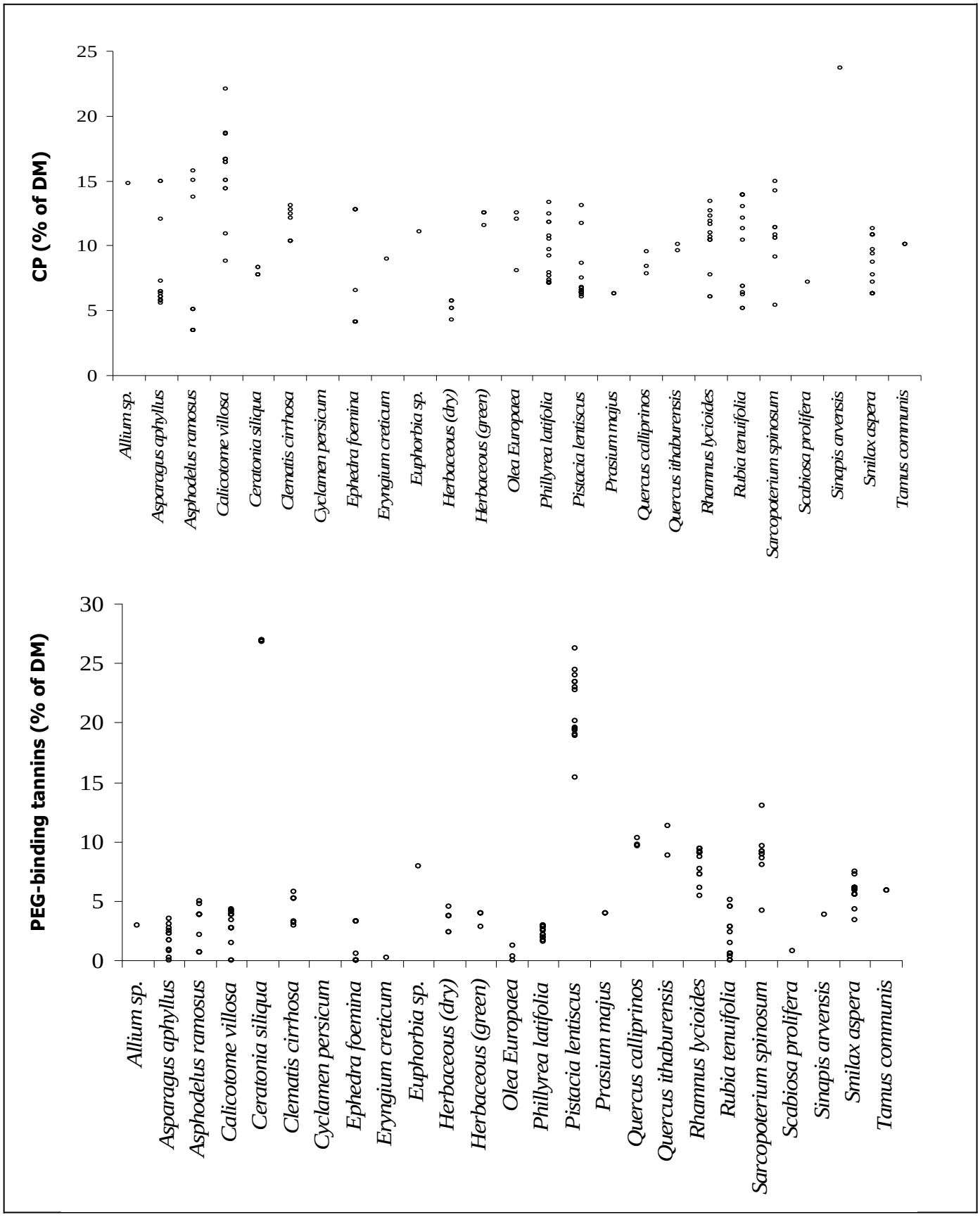

Figure 2. Crude protein (a) and PEG-binding tannins (b) contents (% of DM) obtained for each species for the various bite-type categories and year-season combinations (n = 180). Y-axis: % of DM. Reused by permission of the American Society of Animal Science © 2008.

The successful use of F.NIRS to predict the percentage of leafy spurge (*Euphorbia esula*) in confinement experiments has been demonstrated (Walker et al. 1998). Fecal NIRS also enabled prediction of the botanical composition of individual browse species in mixtures of four species (Landau et al. 2004a), but calibrations based on confinement experiments were not sufficiently robust when applied to free-grazing shrubland conditions (T. Glasser, unpublished), probably because of the complexity of goats' diets. It has to be emphasized that the F.NIRS calibrations developed in the present study are not meant to be used outside of Ramat Hanadiv, Israel.

The R^2 values for the calibrations of *P. latifolia* and *P. lentiscus* obtained in the present study were lower (0.89 and 0.77, respectively; Table 3) than those obtained in well controlled confinement experiments with the same plant species (0.94 and 0.95, respectively; Landau *et al*. 2004a). In the confinement experiments, the SECV values for percentages of *P. latifolia* and *P. lentiscus* (*i.e*., 6.3 and 7.0% of DM, respectively) were similar to the SECV reported in this study. However, the SECV/mean ratio was 15 to 20%, compared with 35 to 65% in the present study. It is probable that some of the difference arose from noise in the fecal spectra generated by the variety of species ingested, and some of it from errors in the estimation of bite number and weight. Also, free grazing animals can be expected to vary their diet botanical composition from day to day compared to pen fed animals that have a constant diet (Campbell et al. 2007). They may do this while maintaining a constant nutrient composition (Kababya et al. 1998), which can explain the higher R^2 for chemical than for botanical composition of selected diets.

The present calibrations for nutritional attributes, at least for CP and *in vitro* DM digestibility as percentages of DM ingested (Table 3), have more general predictive potential according to the criteria of Williams (2001). The R^2 values of our present calibrations for dietary CP and *in vitro* DM digestibility (0.93 and 0.91, respectively) are similar to those that Leite and Stuth (1995) obtained by using grazing, esophageally-fistulated goats (0.94 and 0.92, respectively), but lower than those reported by Landau et al. (2004a) for goats that were hand-fed with browse diets (0.98 for both attributes). The SECV values, relative to the means of the respective nutritional attributes, were low, indicative of satisfactory accuracy.

As reported previously for goats that were hand-fed in confinement with combi-nations of Mediterranean browse (Landau et al. 2004a), F.NIRS calibrations of dietary percentages are more precise and accurate than those of absolute rates of intake. This was expected because NIRS is primarily a methodology aimed at determining chemical composition (*i.e*., percentages). A similar result was reported by Boval et al. (2004) for cattle. Therefore, one would expect to obtain a more accurate estimate of absolute nutrient intake rate by multiplying the dietary percentages obtained from F.NIRS measurements by an independently estimated total DM intake. Calculation of DM intake requires knowledge of fecal output and of the digestibility of a repre-sentative diet. Fecal output can be determined accurately by means of indigestible markers such as chromium sesquioxide (Kababya et al. 1998), a long-chain n-alkane (Decandia et al. 2000), or polyethylene glycol (Landau et al. 2003). Digestibility can also be estimated fairly by F.NIRS (this study). Further research is

needed to verify that F.NIRS can be used to obtain accurate estimates of nutrient intakes when combined with estimates of fecal output using markers.

Collecting reference values for F.NIRS is a labor-consuming task. Many F.NIRS equations rely on fecal samples collected with goats hand-fed diets containing browse (Glasser et al. 2007) or not (Landau et al. 2008) under controlled conditions. Could reference values for botanical composition collected in cafeteria-type experiments be used with free-grazing goats? The answer is, unfortunately, negative. The validations of the dietary percentages of *P. lentiscus* and *P. latifolia* with calibrations established in cafeteria trials were relatively precise (R^2 values of 0.80), but biased (8.6 and 2.2 percentage points, respectively), and more worryingly, slopes differed from unity (1.2 and 0.7, respectively; Glasser et al. 2007). Values for SECV were 16.8 and 13.9 (compared with approx. 6 to 7% using the bite count procedure, see Table 3). The reverse validations also yielded disappointing results. Analyses of the reciprocal distances from each data-set to the centroid of the other clearly showed that the two sets encompassed distinct (Shenk and Westerhaus 1991) spectral populations (Glasser et al. 2007).

Last but not least, in the future, fecal NIRS calibrations based on bite-counts will need to be updated continuously in order to encompass the spectral variety associated with new grazing conditions, as recommended by Coleman et al. (1995) for all NIRS procedures.

ACKNOWLEDGEMENTS

This research was supported by Research Grant No. IS-3555-04 from BARD, the United States - Israel Binational Agricultural Research and Development Fund, and by Texas Food and Fibers Commission Project No. b-04-05-02. ARO Publication no. 136/2005.

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Chapter 7. Fecal NIRS: What Else, What Next?

Doug Tolleson

Objectives: To describe the many ways F.NIRS has been used to find information about diet and other attributes of animals such as sex, species, disease and pregnancy. Advances in instrumentation and the ability to collect information in the field are described.

Key Points

- F.NIRS was first used to predict nutrient then botanical composition of the diet of free-grazing herbivores.
- F.NIRS can classify animals for a variety of attributes such as sex, reproductive status, species and disease status.
- F.NIRS can provide multiple determinations both qualitative (species, sex, reproductive status) and quantitative (diet nutrient content, botanical composition) without expense or stress of gathering animals.
- Tables in this chapter provide a complete summary of studies that have used F.NIRS for qualitative and quantitative findings.
- These findings can be used to make management changes including decisions regarding supplementation or replacing bulls during breeding season.
- Instrument advances will allow management decisions to be made in the field.

INTRODUCTION

Feces is not the most pleasant material on which to base a career, but for those scientists willing to examine it, this material provides a wealth of information. Fecal analysis can be non-invasive, and human doctors often use stool samples in their diagnoses. Wildlife biologists historically have determined diet composition from scat, and old time livestock producers judged diet quality by fecal color or the way the manure of the herd "stacked."

Fecal analysis can include sophisticated techniques such as radioimmunoassay or polymerase chain reaction to evaluate endocrine or disease status. As discussed in the other chapters of this volume, grazing animal diet quality and/or botanical composition have also been determined with fecal near-infrared spectroscopy (F.NIRS). Among agriculturalists these are the more well known applications of fecal analysis techniques.

For the sake of completeness, I will discuss each, but for the sake of brevity, I will not treat either with any depth. The objectives of this chapter are to explore other applications of the technology, increase awareness, and hopefully stimulate interest in further scientific exploitation of F.NIRS by livestock and natural resource investigators.

History and Scope

The application of NIRS on feces for the purpose of measuring diet quality was first reported by Brooks et al. (1984) to determine diet crude protein (CP) and dry matter digestibility as well as dry matter intake (DMI) and average daily gain for pen-fed elk. Although only a small study, these authors illustrated the ability of NIRS on feces to determine herbivore diet or ingestive characteristics. Biston et al. (1988) and Waelput et al. (1990) further explored using NIRS of feces to determine diet characteristics of herbivores. By the time Brook's study was published, work was in progress at Texas A&M University and the USDA-ARS in Oklahoma to develop the F.NIRS technique for grazing cattle. Coleman et al. (1989) represents their initial report on using fecal spectra to predict diet quality in cattle. This work later evolved into that of Lyons and Stuth (1992) which is recognized as the first sufficiently robust calibration to support application of the technique in practical grazing situations. Other workers, primarily but not exclusively with Stuth's lab at Texas A&M, have developed F.NIRS diet quality calibrations for other species and in other locations (Table 1). Botanical composition of herbivore diets is the next most recognized application of F.NIRS. This work has been done primarily in small ruminants by Walker in the US and Landau in Israel. Diet composition of several forage species has been successfully determined using this technology (Table 2). Furthermore, NIRS of feces has been reported for numerous wild and domestic species (Table 3).

For the purposes of this discussion, applications of F.NIRS have been divided into either direct or derivative determinations. As discussed in a previous chapter, direct calibrations are ones in which both reference chemistry and spectroscopy are performed on the same material. Conversely, derivative calibrations are ones in which spectra are collected on a different material (e.g., feces) than the material from which constituent data is collected (e.g., diets). Examples of the direct calibrations are fecal fat or nitrogen (Table 4); examples of the derivative calibrations are dietary CP or percent juniper consumption (Table 5). In addition to direct and derivative calibrations

F.NIRS can be divided into two categories based on the type of determinations, namely: a) numerical determinations, such as those previously discussed, and b) discriminant determinations. Discriminant determinations refer to classifying spectra into qualitative categories such as species or physiological status of the animal from which it was obtained. Table 6 shows the different categories that feces have been successfully classified in using discriminate analysis of fecal spectra.

Table 1. Published research on the development of diet quality calibrations utilizing near infrared spectroscopy of feces.

Reference	Location	Species
Brooks et al. 1984		
Journal of Wildlife Management	Utah, USA	Elk
Coleman et al. 1989		
International Grassland Congress	Texas, USA	Cattle
Gallagher 1990		
Texas A&M PhD Thesis	Texas, USA	White-tailed deer
Lyons and Stuth 1992		
Journal of Range Management	Texas, USA	Cattle
Lyons et al. 1995		
Journal of Range Management	Texas, USA	Cattle
Leite and Stuth 1995		
Small Ruminant Research	Texas, USA	Goats
Whitley 1996		
Texas A&M MS Thesis	Texas, USA	Cattle
Coates 1998		
CSIRO Final report	Queensland, AU	Cattle
Purnomoadi et al. 1998		
Animal Science Technology	Japan	Cattle
Ossiya 1999		
Texas A&M PhD Dissertation	East Africa	Cattle, Sheep, Goats
Kisiksi et al. 2000		
Tropical Grasslands	Queensland, AU	Cattle
Krachounov et al. 2000		
Zhivotnov'Dni Nauki	Eastern Europe	Sheep
Coates 2004		
CSIRO Final report	Queensland, AU	Cattle
Boval et al. 2004		
Animal Feed Science & Technology	Guadeloupe	Cattle
Awuma 2003		
Texas A&M PhD Dissertation	East & West Africa	Cattle, Sheep, Goats
Gibbs 2006		
University of Queensland PhD Thesis	Queensland, AU	Cattle
Li et al. 2006		
Small Ruminant Research	Texas & South Dakota, USA	Sheep
Showers et al. 2006		
Rangeland Ecology & Management	Texas, USA	White-tailed deer
Keating 2005		
Texas A&M PhD Dissertation	Texas & Oregon, USA	Elk
Landau et al. 2006		
Small Ruminant Research	Israel	Goats
Kidane et al. 2008		
Rangeland Ecology & Management	Texas, USA and Kenya	Donkeys

Table 2. Published research on the development of diet botanical composition calibrations utilizing near infrared spectroscopy of feces.

Reference	Location	Species	Constituent
Coates 2004			
CSIRO Final report	Queensland, AU	Cattle	C3:C4 Proportion
Kronberg et al. 1998			
West Sec ASAS	South Dakota, USA	Cattle, Sheep	Ponderosa Pine
Walker et al. 1998			
J Range Mgmt	Idaho, USA	Sheep, Goats	Leafy Spurge
Walker et al. 2002			
J Range Mgmt	Idaho, USA	Sheep	Mountain Big Sagebrush
Walker et al. 2007			
J Animal Sci	Texas, USA	Goats	Juniper
Tolleson et al. 2000b			
1st Natl Conf Grazinglands	Texas, USA	White-tailed deer	Live Oak
Gibbs 2006	Queensland, AU	Cattle	Several Grass & Crop Forages
Univ Qld PhD Thesis			

Discriminant Classifications

It is at this point in our discussion that we delve into most of the "what else" alluded to in the title of this chapter. Discrimination between samples from animals differing in such characteristics as species or physiology has been accomplished with F.NIRS (Table 6). Tolleson et al. (2005) correctly classified the deer species of 87% of the fecal samples from red (*Cervus elaphus*) and fallow (*Dama dama*) deer grazing ryegrass (*Lolium perenne* L.) paddocks using discriminant analysis of fecal spectra. Similar results were obtained by Wiedower et al. (2007a,b) especially between free-ranging gemsbok (*Oryx gazella*) and greater kudu (*Tragelaphus strepsiceros*) in the Edwards Plateau of Texas where 97% of the fecal samples were correctly classified based on their spectra. In Mongolia, discrimination between herbivores with morphologically similar feces resulted in 88, 66, and 98% correct classification between 1) cattle (*Bos taurus*) and yak (*Bos grunniens*), 2) sheep (*Ovis aeries*) and goats (*Capra hircus*), or 3) horses (*Equus caballus*) and khulan or wild ass (*Equus asinus*), respectively (Prince et al. 2007). Male versus female discrimination via F.NIRS has also been reported for several species (Godfrey et al. 2001, Tolleson et al. 2001a, Greyling 2002, Osborn et al. 2002, Tolleson et al. 2005). In addition, Walker et al. (2007) found that fecal spectra differed by sex between animals fed the same diet and these differences could result in significant differences in the predicted juniper in the diet of animals fed the same amount of juniper. Similarly, in a study utilizing pen-fed white-tailed deer, age class (juvenile versus adult) was identified with F.NIRS (Osborn et al. 2002). Most recently, Wiedower et al. (2008) observed differences in fecal NIR spectra between sexes, age classes, and pregnant versus non-pregnant Giant Pandas (*Ailuropoda melanoleuca*). Discrimination of sex in Eastern Grey Kangaroos (*Macropus giganteus*) was not successful, however (Billing, personal communication).

Detection of differences in fecal chemistry between species and sexes does not seem surprising. It is intuitive that divergence in body size, diet selection, dentition, or gut architecture could cause these differences. Perhaps not as obvious are the differences detected via NIRS in feces from animals varying in other aspects of physiology. These differences in fecal chemistry

may result from changes in metabolism and endocrinology, varying levels of endogenous secretions, and or hindgut microbial population dynamics. Successful discrimination between pregnant versus non-pregnant cattle has been reported (Tolleson et al. 2000a, 2001b,c, Omaria et al. 2004) as has lactating versus non-lactating sheep (Godfrey et al. 2001). In addition to pregnancy or lactation, both of which can be considered nutritional stress, animals experiencing some sort of external stressor, and thus altered metabolism as compared to a non-stressed animal, might also be expected to express differences in fecal chemistry. In a summary of five studies, Tolleson et al. (2007a) reported that animals before, as compared to during, tick infestation were successfully discriminated (80% correct classification) although the range of success rate varied from 0 to 100% when all combinations of four calibration sets determined tick status in the remaining validation set. Further work (Tolleson 2007b) indicated that tick status may be indiscernible from the stress of serving as a research subject during intensive blood sampling regimes.

Other disruptions to homeostasis can be detected with F.NIRS. For instance, Johnes disease is the result of infection by *Mycobacterium paratuberculosis* in the small intestine that can create lesions, malabsorption, and wasting (Chacon et al. 2004). Dairy cows which tested positive for the disease prior to expressing clinical symptoms were distinguished (80% correct classification) from those that were negative using F.NIRS (Anderson et al. 2007, Norby et al. 2006). Residual feed intake (RFI) is a measure of feed efficiency that is independent of body weight. Within a cohort, individual animals may gain similar amounts of weight but at significantly different intakes (Herd et al. 2003). Gutiérrez-Bañuelos et al. (2007) indicated that high versus low RFI may be possible to detect in forage fed cattle with F.NIRS.

Table 3. Species in addition to humans for which application of near infrared spectroscopy of feces has been reported.

Domestic [1]	**Wild** [2]
Cattle	White-tailed Deer
Goat	Mule Deer
Sheep	Elk
Yak	Bison
Horse	Caribou
Donkey	Gemsbok
Camel	Greater Kudu
Hog	Roan Antelope
Red Deer	Giant Panda
Fallow Deer	Elephant
Chicken	Kangaroo
Ostrich	Dugong
	Sea Turtle
	Seal

[1] Traditional domestic livestock or others managed as such.
[2] Includes instances of both free-ranging and captive animals.

By now the reader may be asking a question that has often been posed to the author: "What is the purpose for doing these discriminations with F.NIRS?" The question is a fair one. We already have tools to determine pregnancy and disease status, why F.NIRS? Certainly sex and species can be determined visually, especially in farm animals or most large ungulates. Is discrimination of these characteristics just an interesting academic exercise? Perhaps not unexpectedly, I would disagree and offer the following examples. First, recall the report by Prince et al. (2007) from Mongolia in which NIRS was employed to classify morphologically similar feces from large and small domestic ruminants as well as wild versus domestic equines. That partic-

ular application arose from the need for fecal sampling by rangeland vegetation monitoring crews that are untrained in identifying fecal samples by species and/or pressed for time. These individuals cover a great deal of territory during the grazing season and their sampling efforts significantly augment the work of animal nutrition monitoring crews. While collecting a few fecal samples from nearby herds in conjunction with a vegetation transect does not sound too difficult, if absolute sample identification is required several times a day, this task can become time consuming and detract from their primary objective.

Table 4. Constituents determined by direct near infrared spectroscopy in which the chemical reference method and near infrared spectra were obtained on the same material (feces).

Constituent
Moisture/Dry matter
Ash/Organic matter
Nitrogen
Ammonia
Fat/Fatty acids
Starch/Carbohydrates
Acid Detergent Fiber
Neutral Detergent Fiber
Lignin
Bilirubin
Bile acids
Phosphorus
Calcium
Polyethylene glycol

Secondly, if future studies with F.NIRS confirm previous reports (Tolleson et al. 2001b,c) that pregnant versus non-pregnant cattle can be detected at 30 days of gestation, then this technique could be applied as an early pregnancy test in the pasture. For example, halfway through the breeding season, one could sample a sufficient proportion of the individuals in a herd and compare the number of detected pregnant animals to the expected number of pregnant animals based on the calving distribution. Suppose the detected pregnancy rate is less than that expected; would there be value to the producer to know that information during or near the end of the breeding season rather than at weaning, and without having to gather the animals? Yes, if the producer has management options available, such as extending the breeding season (is nutrition a factor, drought, etc…?), or perhaps adding or changing bulls. If these or other options do not exist, the producer will have the ability to make more informed decisions in planning ahead for marketing extra culls, moving non-pregnant animals to a different breeding season, purchasing/retaining replacements, or allowing for pasture deferment for drought or prescribed burning. The value in this type information comes with the fact that these decisions could be made prior to weaning, i.e. not "chute side." The F.NIRS method for pregnancy detection has been on average, 80.0 to 90.0% successful in field validations (Tolleson, personal observation). I submit that to be useful in practical applications, the consistent

Table 5. Constituents determined by derivative near infrared spectroscopy in which the chemical reference method and near infrared spectra were obtained on different materials (i.e., forage and feces).

Constituent
Crude Protein
Acid Detergent Fiber
Neutral Detergent Fiber
Digestibility
Phosphorus
Tannin
Botanical Composition

accuracy should be ≥ 90.0 %. Similarly, if F.NIRS parasite and or disease calibrations continue to be developed successfully, free-ranging livestock and wildlife could be used as sentinels for detecting the distribution of vectors such as fever ticks.

So there is potential for practical application of the F.NIRS discriminant method. Consider also that the F.NIRS technique could be used in a decision tree approach like that reported for NIRS to detect adulteration in meats (chicken, turkey, pork, beef, and lamb: Downey et al. 2000), in which small unknown homogenized meat samples were first identified as being "white" or "red", then if white, as pork or poultry, and subsequently, if poultry, as chicken or turkey. Envision then for our discussion that a researcher or manager was interested in habitat use by several sympatric ungulates, wild and domestic, all with pelleted feces. An experienced individual might easily distinguish between the species and perhaps between males and females simply by physical morphology. Alvarez (1994) used morphology to distinguish between fecal pellets of male and female red and fallow deer. The success rates for visual identification ranged from 60.0 to 80.0% in this study. Perhaps our investigator requires a higher accuracy or wishes to employ untrained technicians for this task. Technicians could opportunistically collect a large number of individual samples, which could then be discriminated at a latter time as described for the meat example (i.e., grouped as to species, followed by sex, and finally, reproductive status [Figure 1]).

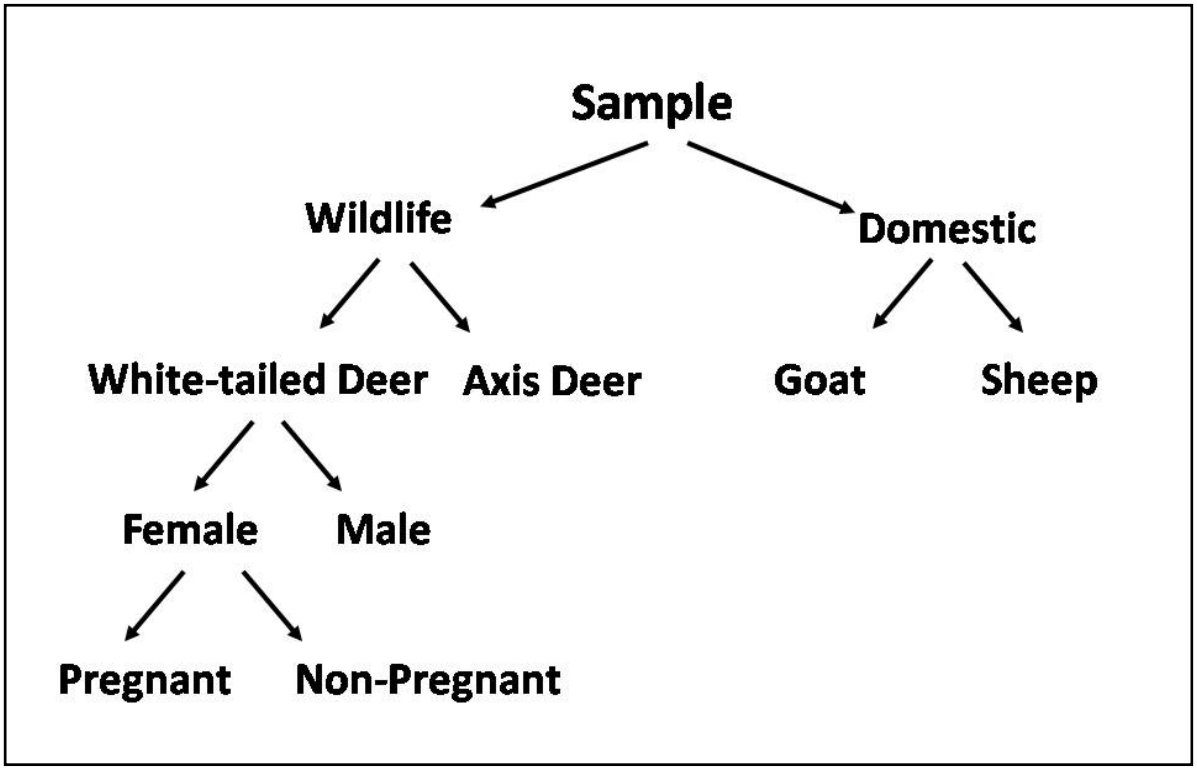

Figure 1. Application of a decision tree approach to fecal NIRS discriminant procedures.

Of course as with all NIRS calibrations, the initial work to establish a discriminant equation sufficiently robust to be useful will be substantial and may be location specific. But savings in time and resources over the long term could be cost effective. Add to this previous example the fact that diet quality or composition could be determined in the same samples and that the samples are not destroyed. Thus, these same samples could be used to obtain information from a chemical reference method for which a NIRS equation does not currently exist. Lastly, as mentioned previously, F.NIRS offers the potential to accomplish all these measurements without the expense or stress of gathering and handling the animals.

Table 6. Physiological characteristics determined by near infrared spectroscopy of feces.

Characteristic
Sex
Species
Age
Lactation
Parasite Status
Reproductive Status
Disease Status
Mineral Status
Feed Efficiency

One recent development of direct F.NIRS is that of quantifying the fuel quality (i.e. BTU's) of feedlot and dairy manures. Waste management is a significant logistic problem for confined animal feeding operations. Development of sustainable bio-fuels is becoming increasingly important to society. Miller et al. (2008) determined DM and OM in manure obtained from feedlots in the Texas panhandle. These values were then input to the regression for prediction of BTU's as reported by Annamalai et al.(1987). The simple coefficient of determination between laboratory and NIRS determined BTU was 0.56 ($P < 0.05$).

The derivative category of F.NIRS can also be sub-divided into those calibrations dealing with a) diet, or b) non-diet characteristics. Arguably, we could add intake to that list of diet characteristics already discussed. The utility of quantifying intake for grazing animals is fairly evident. Numerous authors have reported on this application (Table 7) with variable success. The consistent, accurate determination of intake by free-ranging animals would be one of the most important developments with F.NIRS. Determination of dietary tannin consumption via F.NIRS would also be an important development. Tannins can affect animal performance positively (parasites, bloat, protein bypass) and negatively (digestion, tie up protein). Landau et al (2004) used NIRS to quantify polyethelene glycol binding of tannins in browse (r^2=0.91 sep=1.7%). Ossiya (1999) and Tolleson et al. (2000b) have completed preliminary efforts toward measuring tannins in herbivore diets using F.NIRS. Ingestion of harmful plants could be another application of F.NIRS. Identification of feces from cattle grazing endophyte-infected fescue (*Festuca* spp.) versus non-infected and "novel" infected varieties was accomplished by Andrae et al. (unpublished). In the non-diet category, Gibbs (2006) has reported that F.NIRS has been used to predict daily fecal (R^2 = 0.80, SEC = 1.2 g DM/kg live wgt/d) and urine (R^2 = 0.70, SEC = 8.0 ml/kg live wgt/d) output in cattle.

Future Directions

Perhaps we will someday be able to look at NIR spectra and directly make determinations about those samples or the animals from which they came. As an example, I will use the data from Tolleson et al. (2000b). In this study, 18 fecal samples

were obtained from white-tailed deer fed diets of either 10 or 20% CP. Within each level of CP, 3 levels of oak leaves (none, low, high) were offered.

Table 7. Determination of intake in grazing animals by near infrared spectroscopy of feces.

Reference	Species
Brooks et al. 1984 J. Wild. Mgmt.	Elk
Coleman et al. 1989 Intl. Grassland Congr.	Cattle
Gallagher 1990 TX A&M MS Thesis	WT Deer
Coleman et al. 1995 Symp: Intake by Feedlot Cattle	Cattle
Coates 1998 CSIRO Final report	Cattle
Tolleson et al. 2002 Soc. Range Mgmt. (abs)	Cattle, Sheep, Elk
Coates 2004 CSIRO Final report	Cattle
Boval et al. 2004 Anim. Feed. Sci. & Techn.	Cattle
Gibbs 2006 Univ Qld PhD Thesis	Cattle

Figure 2 illustrates the average NIR spectra for each group. Some differences in spectra between groups are evident in these linear graphs. What is more interesting though is the examination of these spectra with principal component (PC) analysis (Figure 3). This dataset provides a classic example of the differences between fecal chemistry attributed to diet and how different diet constituents affect fecal spectra in different ways.

Similar relationships may exist for differences in fecal spectra due to sex or species for instance. In the aforementioned example, notice that CP is described by PC axis 1 and that oak leaf proportion is distributed along PC 2 and 3. If the analyst had only performed reference chemistry for protein, and not "looked" at the spectra, these samples would only be considered as high and low protein. Near infrared spectra contain more information about the physico-chemical properties of a substance than that extracted via regression with a single or combination of reference methods. Thus, the creative application of chemometrics could be applied to glean more information from a single sample than is currently routinely accomplished. In what other areas of interest could this previous example or similar techniques be exploited?

Which brings us to "what next"? All of the previous discussion has involved F.NIRS in which spectra were collected on a static bench-top spectrometer. This process involves time to collect a sample, bring it to a lab and then process, scan, and apply a predictive equation. While this procedure is often more rapid than conducting most typical chemistry analyses, the utility of these measurements for both research and practical management will increase dramatically if the technique could be done in the field, in real time. Work is currently underway to develop the capability of portable NIRS for a "take the lab to the sample" approach (Figure 4).

Tolleson and Stuth (2005) reported that portable NIRS had the ability to re-create established diet quality calibrations for sheep. We also collected portable NIR spectra on sideoats grama (*Bouteloua curtipendula*) and goat fecal pellets in the pasture with similar repeatability to that

obtained on a static machine (Tolleson and Stuth 2006). The portable NIRS technique has not only been employed in the analysis of feces, but also to categorize mohair fiber on live animals (Prince et al. 2007) and determine various differences in samples of blood or milk (Tolleson, personal observation).

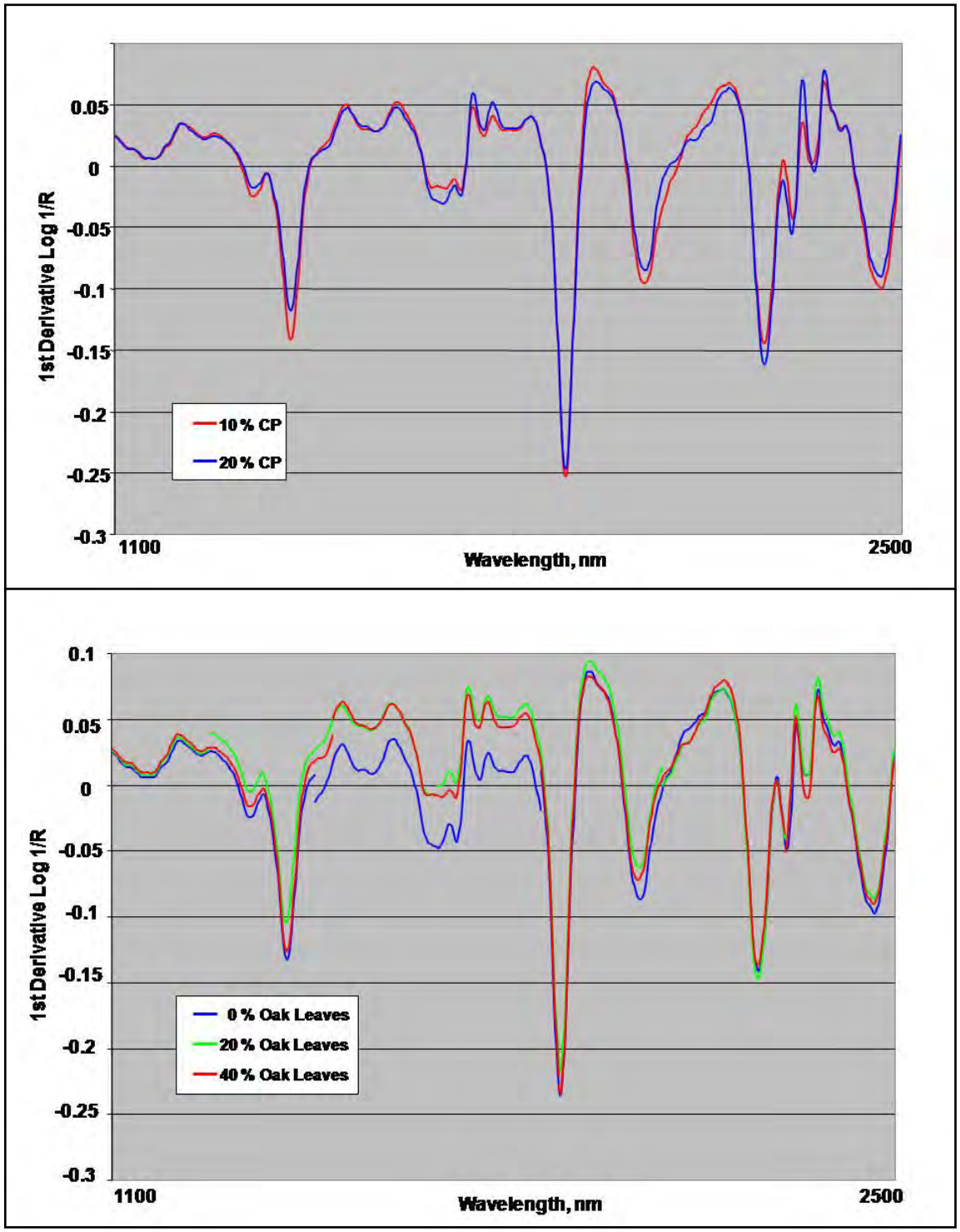

Figure 2. Effect of dietary oak leaf and crude protein content on fecal NIR spectra of white-tailed deer.

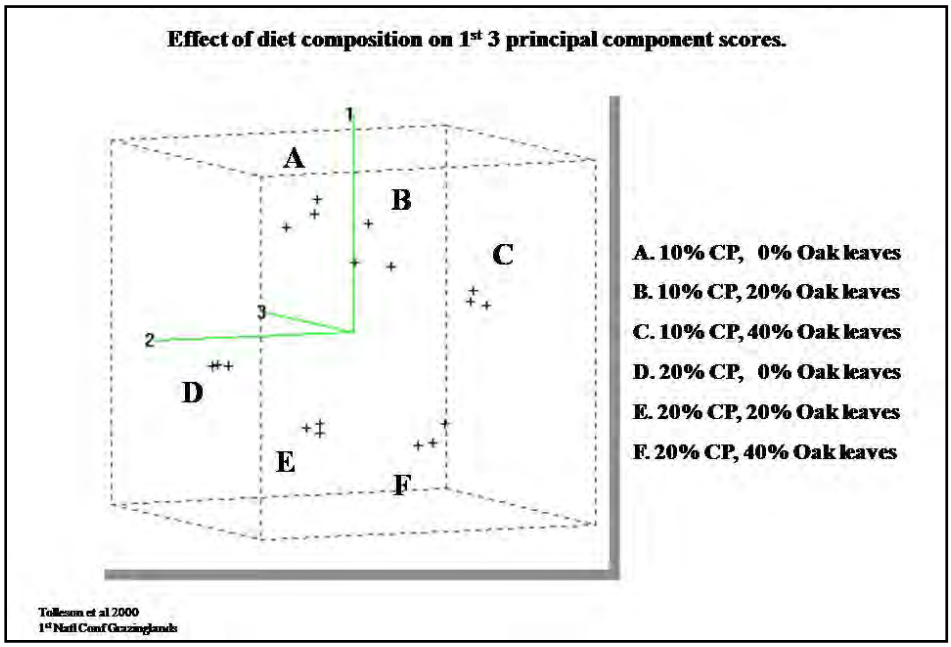

Figure 3. Fecal NIR spectra of white-tailed deer.

SUMMARY and CONCLUSIONS

In conclusion, I will leave you with these questions pertaining to the method of NIRS of feces: 1) What else can be done or improved with this technology as it is presently applied and, 2) What haven't we done that could be done, i.e. what next? I submit that we, as animal and range scientists, have just begun to explore, develop and perfect this technology. As evidenced by this entire publication, much has been accomplished in the field of F.NIRS, and in a relatively short time. There have been successes and failures. But there is so much more to be done.

Figure 4. Application of portable NIRS of feces in the field.

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Technical Bulletin
SANG-2010-0250
July 2010

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Society for Range Management